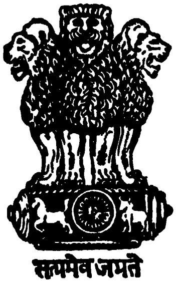

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ON THE ORGANIC BASES OF THE PUPA OF TUSSAH SILK WORM (ANTHERAEA PERYRI).

By Jirō KATŌ.

From the Central Laboratory, South Manchuria Railway Co., Dairen, South Manchuria.

(Received March. 8th., 1926)

4 kgs. of powdered pupa of tussah silk worm were extracted with hot water. About 40 litres of the aqueous extract thus obtained were evaporated to about one-tenth of its volume. The precipitate formed on this process consisted mainly of the urate, as was reported previously (The report of the coutral laboratory of S. M. R. Co., No. X.) It was filtered and washed with water. The filtrates and the washings were combined and treated by lead acetate to get rid of impurities. From the filtrate, the lead was removed by passing sulfuretted hydrogen. When the filtrate was neutralized and evaporated down to about 2 litres, about 2 grs. of tyrosine crystallised out. The filtrate from tyrosine was treated with phosphotungstic acid and baryta in the ordinary way.

The free base solution thus obtained was separated into the following fractions :- purine fraction, histidine fraction, arginine fraction and lysine fraction. From these fractions the following bases were isolated :-

Adenine as picrate.	2.8 g.
Hypoxanthine.	0.27 g.
Histidine as dipicrolonate.	0.30 g.
Arginine as picrate.	0.10 g.
Choline as gold salt.	8.50 g.
Betaine as picrate.	present.
Lysine as picrate.	9.51 g.

The content of the bases of this pupa is smaller than that of the silk worm pupa. Any special base, which has not been isolated from the silk worm pupa, was not detected. The cadaverine and putrescine, which K. Katayama isolated from the silk worm pupa, were not met with here. The absence of putrescine and cadaverine, the greater yield of lysine, and the smaller total content of organic bases are the chief difference of this pupa from the silk worm pupa, in regard to organic bases.

THE CHEMICAL PROPERTIES, AND THE NUTRITIVE VALUE OF THE PROTEIN OF ITALIAN MILLET (SETARIA ITALICA, KTH).

By Mitsuyuki KONDO.

From the Imperial Government Institute of Nutrition, Tokyo,
Dr. Tadasu Saiki, director.

(Received Feb. 13 th., 1926)

In our country, Italian millet is an important cereal, and ranks next to rice and wheat. In spite of this fact there seems to be lacking a detailed investigation of its protein and of its nutritive value.

The Italian millet seed used in these experiments was polished by the polishing machine of this institute and then powdered.

The analysis gave the following constitution :-

	Air dry matter %	Dry matter %
Moisture	13.57	—
Crude protein	11.12	12.27
Protein	10.36	11.98
Crude fat	5.32	6.16
N-free-extract	63.78	73.79
Crude fibre	1.71	2.01
Crude ash	0.87	1.01

1. Solubility of proteins.

Separation of proteins :- before separating the proteins into fractions, their solubility in different solvents was examined. As solvents, distilled water, 10 % saline solution, 0.2 % alkaline solution, and 10 % alcohol were used. The procedure was as follows :-

15 gr. of the sample were agitated with nearly 800 c.c. of distilled water for 24 hours at room temperature. After filtering and washing, the filtrates and the washings were combined and made up to 1 liter. Then the residue was treated with 10 % saline solution, 0.2 % alkaline solution and 70 % alcohol successively in the same way as stated above. Of these four filtrates, the albuminous nitrogen was determined.

	Dry matter %	Total N %
Total N of this sample	1.98	100.00

Water soluble N	0.37	21.85
10% saline solution sol. N	0.43	25.18
0.2% alkaline " " "	0.52	30.15
70% alcohol " " "	0.96	56.21

Subsequently, the samples were extracted by these solvents separately and the following results were obtained :-

Sample gr.	Total N gr	Solvent (200c.c.)	Temp.	Time hrs.	N-extracted gr.	%
3.06	0.0544	Water	40°C	5	0.0228	41.91
2.64	0.0169	10 % saline solution	40°	5	0.0114	24.48
3.81	0.0678	0.2 % alkaline "	40°	5	0.0275	40.62
2.32	0.0413	70 % alcohol "	60°	5	0.0209	50.60

Note: These extractions were made under an elevated temperature.

From these results, it is evident that Italian millet contains mainly the 70 % alcohol soluble protein and 0.2 % alkaline soluble protein, so the author then separated these two proteins.

2. The separation of the alcohol soluble protein.

300 gr. of the sample were heated with 5 times its weight of 70 % alcohol in a triple necked flask and agitated for 6 hours at 60°C and immediately filtered. The yellow filtrate was evaporated in a vacuum to get rid of the greater part of the alcohol. The residue, on pouring into cold water gave the precipitation of the protein as a yellow viscous mass. Then after filtering and washing with water, it was dried, powdered and washed with ether. 18 gr. of nearly pure protein were obtained.

3. The separation of alkaline soluble protein.

350 gr. of the sample were mixed with 4 liters of 0.2 % NaOH solution and agitated for 5 hours at room temperature, and allowed to stand for a few hours. In order to effect the filtration as quickly as possible, it was separated by a centrifugal machine (Sharples), and the filtrate was neutralized with acetic acid. The precipitate thus formed was collected, again dissolved in an alkaline solution and precipitated by acetic acid. After washing with water, alcohol, and ether, the dried protein thus obtained weighed 20 gr.

THE NITROGEN DISTRIBUTION IN ITALIAN MILLET, IN ITS ALKALINE SOLUBLE PROTEIN, AND ALCOHOL SOLUBLE PROTEIN.

1. The nitrogen distribution in Italian millet.

The nitrogen distribution of the seven groups of amino acids in this sample was determined directly by the Van Slyke method as follows:

	Dry matter %	Total N %
Total N	2.033	100.01
Hot 20 % HCl soluble N	1.958	96.29
" " " insoluble N	0.075	3.71
Humin N	0.052	2.54
Amide N	0.244	12.03
Phosphotungstates N	0.605	29.78
Cystine N	0.045	2.21
Arginine N	0.197	9.71
Histidine N	0.210	11.81
Lysine N	0.123	6.05
Amino N	0 508	25.02
Non-amino N	0.097	4.76
Mono amino N	1.003	49.43

2. The nitrogen distribution in the alkaline soluble protein.

The method used was the same as in the previous analysis. The nitrogen distribution in this protein was as follows :–

	Dry matter %	Total N %
Totel N	10.020	100.00
Hot 20 % HCl soluble N	5.681	59.69
" " " insoluble N	4.039	40.31
Humin N	0.555	5.54
Amide N	1.162	11.60
Phosphotungstates N	2.373	23.68
Cystine N	0.188	1.88
Arginine N	0.703	7.02
Histidine N	0.944	9.42
Lysine N	0.537	5.36
Amino N	1.794	17.90
Non-amino N	0.579	5.78
Mono-amino	5.316	53.05

3. The nitrogen distribution in the alcohol soluble protein.

	Dry matter %	Total N %
Total N	13.120	100.00
Hot 20 % HCl soluble N	12.372	94.30
" " " insoluble N	0.748	5.70
Humin N	1.452	11.07
Amide N	2.354	17.94
Phosphotungstates N	1.846	17.94
Cystine N	0.218	1.66
Arginine N	0.664	5.06
Histidine N	0.749	5.71
Lysine N	0.615	4.69
Amino N	1.249	9.52

Non-amino N	0.997	7.60
Mono-amino N	6.879	52.43

From these results, the Italian millet protein is rich in hexone base-N, and also contains an adequate quantity of lysine N. It is therefore to be regarded to have a great nutritive value.

FEEDING EXPERIMENT ON THE NUTRITIVE VALUE OF THIS PROTEIN.

1. Preparation of the ration.

In order that the ration may contain only the protein content of Italian millet, the millet flour was used, and the deficient constituents other than protein were added, using the analysis of the whole grain as a standard. The deficiencies were supplemented as follows: carbohydrate with starch, fat with butter, which is simultaneously used as a source of vitamin A, and mineral matters with Nelson's salt-mixture, and as a source of vitamin B, a small quantity of oryzanin powder was added. The ratio of the constituents of this ration is as follows :-

Protein	10
Fat	14
Ash	4
Carbohydrate	72

Taking the moisture into consideration, the ration was mixed as follows :-

Italian millst powder	100 gr.
Butter	12 〃
Nelson's salt-mixture	4 〃
Starch	10 〃

This diet was heated with four times its weight of water on the water bath until dextrinized.

2. The course of the feeding experiment.

5 male and 5 female albino rats were fed on this diet from Dec. 3, 1923 to Aug. 7, 1924.

It is evident that this diet containing 10 % protein is sufficient to maintain the normal growth of the rats, though there were some increases and decreases of body weight during this period. The average body weight of the young male rats was 58 gr., and this gained the body weight of 306 gr.; while the young female rats increased from the average weight of 59 gr. to 253 gr.

Notwithstanding the fact that the protein of Italian millet is vegetable protein, still it proves to be very favorable for growth.

STUDIES ON THE CELLULOSE.

(PUBLICATION ON CELLULOSE NO. I.)

By Arao ITANO.

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(Received April 28th., 1926)

This paper presents a preliminary report on the bio-physico-chemical problems which are in course of investigation on the cellulose.

Cellulose is one of the most important carbohydrates from many standpoints, namely its wide distribution in nature and its usefulness in manufacturing. Especially in agriculture, the important rôle in maintenance of proper soil conditions has been well known. It however has been very little known as to its exact chemical nature on account of its peculiar properties. Through the thermodynamical studies together with the investigations on the process of decomposition, some additional information may be obtained.

Cellulose is decomposed by comparatively few microorganisms in spite of the enormous amounts of cellulose produced and destroyed every year on earth. Among the few organisms isolated, the anaerobic grouph has been investigated(1) to some extent as to their activity. But the others received comparatively little attention in recent years.(2) An aerobic, thermophilic cellulose fermenter has been investigated here in regard to their energetics, intermediate and end-products of the fermentation.

In regard to the studies of energetics on the members of Schizophyta, only few literature is available. Since Rubner(3) investigated the subject, there has been very little work done. Especially on the soil microorganisms, only few investigation are found on record.(4)

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(1) Omelianski, Centralbl. f. Bakt., II, 1902, 193.

(2) C. van Iterson, Centralbl. f. Bakt., II, **23**, 689;
Hutchinson & Clayton, J. Agr. Science, **9**, 143;
McBeth & Scales, U. S. Dep't Agr. B. P. I. Bull., 266;
Viljoin & others, J. Agr. Science, **16**, 1926, **1**: and others.

(3) M. Rubner, Archiv. f. Hygine, **48**, 1904, 260. **57**, 1906, 161.
A. Putter, Vergleichende Physiologie, 1911, **37**.

(4) S. Winogradsky, Bot. Zeitung, **45**, 1187, 489;
N. L. Sohngen, Centralbl. f. Bakt., **15**, 1906, 513;
F. H. van Suchtelen, Centralbl. f. Bakt., **2**, **58**, 1923.

I. THERMODYNAMICS INVOLVED IN THE CELLULOSE DECOMPOSITION.

As it is well known, the plants synthesize the cellulose out of carbon dioxide and water, and when the plant residues find their way into the soil, they are decomposed by,

1. the enzyme cytase,
2. the anaerobic microorganisms,
3. the aerobic microorganisms.

Of course in the last two cases, the action may be due to the enzyme secreted by the organism, but it has not so far been demonstrated. Again it is not probable that the cytase survives the temperature of 65°C for so long time as the thermophilic cellulose fermenter do unless some special conditions exist. As the end products of decomposition, the carbon dioxide and water are given off. This transformation involves not only bio-chemical but physical process namely the thermodynamics or energetics.

Then, in the process of formation of cellulose, certain amount of energy absorbed or endothermic reaction takes place, and the exothermic reaction follows in course of decomposition. This transformation of energy taking place in soil is very important from the soil microbiological standpoint. That is while the plants utilize the sun's energy in the process of synthesis, the microorganisms must find the energy supply to elsewhere, and the source became more clearly understood by the study of fermentation by Rubner(3) and few others.

In investigating this phase of problem on the cellulose, it was found very difficult on account of the lack of exact chemical knowledge of cellulose and also the nature of the process involved. For instance, in looking up the literature on the heat of formation and also the heat of combustion, the numerical value given by the different authors is somewhat different. Examining the data collected in the light of energy equation of cellulose and comparing it with that of glucose, one finds that

For

$$\begin{aligned}\text{Glucose,}\quad & C_6H_{12}O_6 + 12\,O = 6CO_2 + 6H_2O + 677.2\ \text{Kcal}^{(5)}\\ & -x \quad - \quad 0 \quad = -565.8 - 409.8 + 674. \ \ '' \ ^{(6)}\end{aligned}$$

and for,

$$\begin{aligned}\text{Cellulose,}\quad & C_6H_{10}O_5 + 12\,O = 6CO_2 + 5H_2O + 680.4\ \text{Kcal}^{5)}\\ & -x \quad - \quad 0 \quad = -565.8 - 341.5 + 680.0 \ \ '' \ ^{(6)}\end{aligned}$$

From these equations, the heat of formation(7) is calculated and found as follows: i. e., calculating for x,

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(5) R. Biedermann, Chemiker-Kalender.

(6) W. Nernst, Theoretical Chemistry, 6th edition ;

(7) F. H. Getman, Outlines of Theoretical Chemistry, 3rd Edition.

For
Glucose, 301.6 and Cellulose, 227.3 Kcal.

From the heat of formation thus calculated, one may be informed very approximately as to the quantity of energy involved in the synthesis, and also from the heat of combustion, we find the maximum energy could be liberated on its complete oxidation. Further, from the calorific value determined, the constitution of cellulose may possibly be ascertained more accurately and clearly than has been known.(7) This phase of the investigation will be reported in detail in near future.

In the light of the energy equation, the possible process of cellulose decomposition may be noted as follows, assuming that an initial step is hydrolysis:

$$O_6H_{10}O_5 + H_2O = C_6H_{12}O_6 + x \text{ cal.}$$

In the above equation, the value for x cal. would be very small, and the nature of $C_6H_{12}O_6$ resulted differ by different process. It can be any one of the monosaccharide, namely mannose, galactose, glucose etc. So far as the author is aware, the products of aerobic, cellulose decomposition have not been studied to any extent.

Once the cellulose is hydrolysed into monosaccharide, it offers many possibilities, such as well known reaction, intermolecular etc.,

By an intermolecular reaction:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 22 \text{ Kcal.}^{(3)} = 2C_3H_6O_3 + 15 \text{ Kcal.}^{(3)}$$

By further oxidation:

$$C_2H_5OH + 2O = CH_3COOH + H_2O + 115 \text{ Kcal.}$$

Besides these well known, possible reactions, the products produced first may undergo the further decomposition in course of investigation. Consequently the exact processes, and the nature of the products in the cellulose decomposition may never be found out exactly. However an attempt is made here to study the products of aerobic decomposition and thermodynamics involved, and it is hoped to obtain some additional information on the subject, which will be reported in near future.

II. THERMOPHILIC CELLULOSE FERMENTER.

An organism which has received a special attention in the investigation here is an aerobic thermophilic bacteria of which description will appear later.

This organism has been investigated in view of the fact that the aerobic cellulose fermenter has received very little attention in past, although I believe it plays a very important rôle in the process and subsequently in agriculture. Thermophilic nature of this organism enables it to work at high temperature which is often reached in composting, where the temperature rises up to 75°C often.

III. PRACTICAL APPLICATION.

In recent years, the study on the rôle of organic matter in soil fertility has become acute.(8) Schreiner(9) stated "It is only by continually supplying organic matter that the soil-forming, soil fertility promoting, dynamic changes can continue to go on unchecked and undiminished, liberating ammonia and other compounds, *supplying energy* for bacterial life and furthering nitrification and nitrogen fixation". Further the same author stated, "If we would understand soil fertility as influenced by *organic manures*, green manures, and good farming methods we must study not so much the organic content, except it be as a key to these *dynamic factors*, but the organic chemical changes themselves which affect soil fertility must be clearly worked out. In this field of research activity much remains to be done".

While the scientific investigations named previously have been in progress, the practical experiments have been carried out during the last one year and half to obtain a desirable organic farm-yard manure, or composting on the basis of scientific information available. A brief abstract of the method will be given below and the detail description will be published later:

1. The materials used;

   Straw, weeds, garbage, street sweeping, plant residue, rice hask, human manure, and any other organic waste materials may be used.

2. The zymotic chamber;

   The chamber is so constructed that permits as much oxidation as possible to take place in course of fermentation. The use of thermophilic fermenter is made freely in case it is necessary.

3. The products;

   The content of the chamber is taken out after the temperature falls down and becomes constant, on average, it requires about three weeks. The compost thus produced seems to be well fermented as that produced by an ordinary method of composting which requires much longer time. The chemical composition of the product varies as the initial materials which are put into the chamber vary. An average of some samples were produced from rice straw, barley straw (fresh and some used once on roof), rice hasks, and weeds, gave the following composition.

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(8) Symposium on "Soil Deterioration", J. Amer. Soc. of Agronomy, 18, 2, 1926.

(9) O. Schreiner, ibid, p. 121.

	Percentage.
Total nitrogen,	2.00
〃 potassium,	1.44
〃 phosphates,	0.85

IV. SUMMARY AND CONCLUSIONS.

1. In the field of dynamic studies of organic matter especially of cellulose in agriculture, more extensive as well as intensive research investigation should be carried out.

2. The thermodynamical study in the general microbial processes specially on the soil microorganisms should be investigated in order to obtain better knowledge of soil fertility.

3. The thermophilic cellulose fermenter seems to act vigorously on the cellulose in course of composting as well as on highly refined cellulose in culture medium.

4. A specially constructed zymotic chamber seems to aid in producing the desirable compost out of various waste meterials in comparatively short time without the aid of cattle.

5. Such method of composting may be employed in a large scale as a process of disposing the waste materials in city as well as on the farm.

STUDIES ON THE RELATION OF BLOOD CONSTITUENTS AND FLACHERIE IN SILK-WORMS.

By Otomatsu FUJII.

(Received Feb. 22nd., 1926)

I. QUANTITATIVE CHANGES OF THE BLOOD CONSTITUENTS DURING THE DEVELOPMENT OF SILK-WORMS.

1. The main purpose of this research is to investigate the causes of flacherie in the silk-worm, from the biochemical point of view. It is a well known fact that flacherie always appears at some definite periods, as for example one or two days after the fourth sleep and at the most active period

of feeding in the fifth age. So that, as a preliminary test, the author carried out a series of experiments to discover the changes occurring in some of the blood constituents during the development of the silk-worm.

In the blood constituents, total N, protein N, organic base N, amino acid N, P, Cl, Mg, K, and Ca were determined and also PH was investigated. As the result of these experiments it became clear that the concentration of these constituents generally increases in the blood according to the increase of the amount of food digested, whilst the amino acid N alone decreases in the fifth age.

2. The author proposed a certain modification of Folin and Wus' method (J. Biol. Chem., **38**, 81, 1919) for the determination of nitrogen in the blood and discussed the sources of errors in the method.

II. ON THE CAUSE OF FLACHERIE.

Flacherie, a disease of the silk worm, has been investigated by Pasteur, Vernon, Omori, Honda, Chigasaki etc. from the bacteriological point of view, and many species of bacteria have been isolated from the blood and the intestines of the diseased bodies, but further investigations have shown that these bacteria could not multiply in the blood or intestines of the healthy worms, and many trials have failed to produce this disease in the worms by feeding them with mulberry leaves on whose surface bacteria had been artificially smeared. On the other hand it has been reported by Cuboni, Voglino, and Sawamura that these bacteria are always found on the natural mulberry leaves. Hitherto the failure in hygienic cares, such as temperature, moisture and the method of rearing, were accounted as the cause of flacherie, but it was shown by Okushi (Report of Kumamoto Sericultural Experimental Station V, I, No. 3) that the variation of temperature and moisture in the natural state have no such effect on the worms.

The author, therefore, attempted to ascertain the cause of this disease from the biochemical viewpoint, and first of all studied the quantitative difference in some of the blood constituents between healthy and diseased bodies. According to the results of these experiments the amounts of protein N, amino acid N, Ca, Mg and Cl are greatly diminished in the blood of diseased bodies, as compared with those in the normal blood, and this abnormal condition agrees with that which arises in the healthy worms of the fifth age when they had been starved for forty eight hours. Such starved worms do not show any sympton of flacherie. But, when these starved worms were fed with mulberry leaves on whose surface some of the bacteria (or the blood of diseased worms) were smeared, about 90 % of them developed the full symptoms of flacherie.

From the above results, it seems therefore that the infection with the bacteria alone can not be the cause of the disease, but that the disturbances in the digestive organs must go first and give chance to the rapid multiplication of bacteria, once infected, in the intestinal canal and the blood. The fact that the respective bacteria are always present on the mulberry leaves also confirms this conclusion.

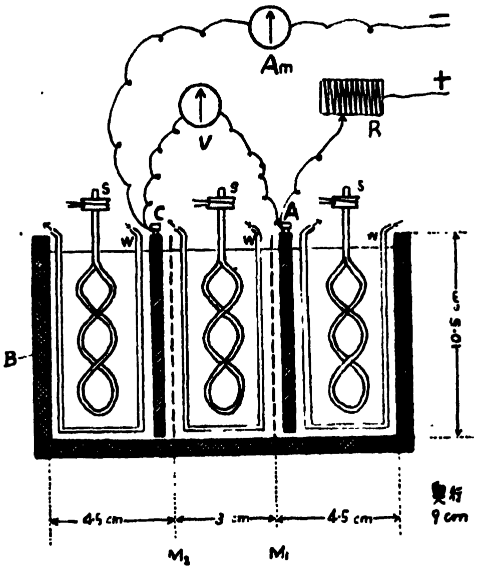
THE SEPARATION OF DIBASIC AMINO ACIDS BY ELECTROLYSIS.

Tarō Noguchi.

(Received March 21st., 1926)

Experiments were carried out with the neutral barium salt solution of both pure glutaminic acid and aspartic acid, placing one of these solutions in the center of a three compartment cell and distilled water in the end compartments as shown in the figure, using carbon electrodes. The operation was made in various conditions, and the results obtained were as follows: Almost no destructive decomposition occurred when the current density at the anode surface did not exceed 0.25 amp. per 100 sq. cm. If the concentration of the amino acid wandered in the anodic compartment was kept under 0.5 % and the temperature in each compartment was maintained at 25°C by means of the water coolers, 90–95 % of the sample substance in the center. compartment migrated through the membrance to the anode. In this case, a parchment paper membrane on the cathode side and a gelatin membrane on the anode side were found most suitable for keeping the solution in the center compartment neutral. Both the glutaminic and aspartic acids obtained in the anodic compartment were purified and analyzed.

By using the above method aspartic acid was separated from such a rejected liquid, which had been produced abundantly in our laboratory by hydrolyzing the protein of the soy-bean with sulphuric acid, removing the glutaminic acid present as calcium salts, and subjecting the resulting liquid to the butyl alcohol extraction. Thus 6.4 liters of the remaining liquid of the butyl alcohol extraction were electrolyzed, using a somewhat larger apparatus, and 75 g. aspartic acid were obtained after 36 hours of electrolysis. The acid was then recrystallized and analyzed.



B.	Bath (Cell)
A.	Anode
C.	Cathode
M_1	Gelatine membrane
M_2	Parchment paper
S.	Stirrer
W.	Water cooler
V.	Voltmeter
Am.	Ammeter
R.	Resistance

STUDIES ON ACIDS FORMED BY RHIZOPUS SPECIES. PART II.

FORMATION OF ETHYL ALCOHOL FROM TARTARIC OR FUMARIC ACID.

By Teizo TAKAHASHI, Kinichiro SAKAGUCHI and Toshinobu ASAI.

(Received July 15th., 1925)

Further five species of Rhizopus of various origin were studied. One of them, Rh. japonicus Saito, formed mainly fumaric acid with trace of lactic acid, while Rh. shangheiensis Yamazaki gave both these acids in a reversed proportion. The other three species, Rh. nodosus Yam., Rh. Batatas Nakazawa, Rh. Tritici Saito formed both lactic and fumaric acids.

Lactic acid formed by these strains was *l*-lactic acid as we have mentioned in the previous report.

In the volatile products there were found beside ethyl alcohol both formic and acetic acids. The quantitative determination of acetic acid was made of this distillate after the destruction of formic acid by K–bichromate and sulphuric acid. The quantity of formic acid was calculated from the difference of the total acidity and that due to the acetic acid.

In a previous report the presence of malic acid was mentioned from the result of qualitative test. This acid has now been isolated and accurately identified by Dakin's⁽¹⁾ method. Cinchonin-*l*-malate thus obtained melted at 197–198°C (uncorr.), as given by Dakin.⁽²⁾

The results of the analyses are shown in the following table :–

Species of Rhizopus.		R. japonicus	R. nodosus	R. Batatas	R. Tritici	R. shanghaiensis
Weight of fungus in 1 L. medium.		1.109 gr.	1.320	1.268	1.455	1.002
Sugar consumed.		38.4 gr.	47.5	46.8	37.8	54.0
Acidity of medium expressed in c.c. of $^1/_{10}$n NaOH to neutralize 10 c.c. of it.		14.4 c.c.	22.2	24.6	18.6	21.6
Quantity of acid ppted as Pb-salt in the residue of ether extract expressed in c.c. of $^1/_{10}$n NaOH.		32.8 c.c.	6.8	3.3	24.2	4.9
Quantity of volatile acid from 1 L. medium expressed in c.c. of $^1/_{10}$n NaOH.	Formic acid.	1.0 c.c.	7.8	2.0	13.4	2.3
	Acetic acid.		12.2	8.0	11.6	1.7
Alcohol formed (wt. %)		0.67%	0.16	0.29	0.54	0.23
Acid in ether extract from 1 L. medium.	Fumaric acid.	6.07 gr.	0.105	0.059	0.041	trace
	Lactic acid.	trace	17.1 gr.	18.0	13.9	16.5
Zn-lactate	Water of crystallisation.		13.05%	13.14	12.91	13.21
	$[\alpha]_D^{t^\circ}$		+ 6.75°	+ 7.1	+ 6.81	+ 6.85
Remarks		Ba-salt, soluble in 80% alcohol, obtained from the ether extract was 0.03 g. Malic acid obtained as cinchonin-salt was 0.02 g.				Ba-salt insoluble in 80% alcohol obtained from the ether extract weighed 0.36g.

The formation of lactic acid and alcohol from fumarate was affirmed in in the culture of Rh. G. 36 in the medium consisting of: water 100 c.c., peptone 0.3 g. and K–fumarate 2.5 g. besides mineral matters. Lactic acid amounting to 0.063 g. was determined by Ripper's method.⁽³⁾ The quantity of ethyl alcohol was too small to be determined quantitatively althongh it

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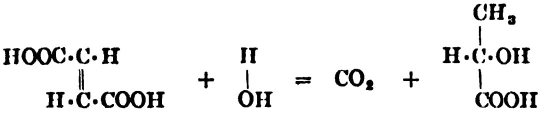
(1) and (2). Dakin: Journal of Biol. chem. Vol LIX, No 1, p. 7. 1924.

(3) Ripper: Bioch. Zeit. **42**, S. 91–104, 1912.

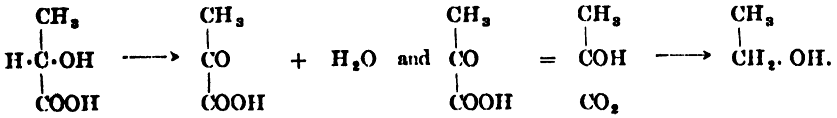
gave iodoform when treated by iodine and sodium hydroxide. The change of fumaric acid into lactic acid may be shewn in accordance with equation :-



$$\begin{array}{l}\mathrm{HOOC{\cdot}C{\cdot}H}\\ \quad\;\|\\ \mathrm{H{\cdot}C{\cdot}COOH}\end{array} + \begin{array}{c}\mathrm{H}\\ |\\ \mathrm{OH}\end{array} = \mathrm{CO_2} + \begin{array}{c}\mathrm{CH_3}\\ |\\ \mathrm{H{\cdot}C{\cdot}OH}\\ |\\ \mathrm{COOH}\end{array}$$

[I]

As to the production of ethyl alcohol the most probable explanation is the intermediate formation of pyruvic acid from lactic acid, as already proved by Kayser(4) in the case of yeasts.



$$\begin{array}{c}\mathrm{CH_3}\\ |\\ \mathrm{H{\cdot}C{\cdot}OH}\\ |\\ \mathrm{COOH}\end{array} \longrightarrow \begin{array}{c}\mathrm{CH_3}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{COOH}\end{array} + \mathrm{H_2O} \text{ and } \begin{array}{c}\mathrm{CH_3}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{COOH}\end{array} = \begin{array}{c}\mathrm{CH_3}\\ |\\ \mathrm{COH}\\ \\ \mathrm{CO_2}\end{array} \longrightarrow \begin{array}{c}\mathrm{CH_3}\\ |\\ \mathrm{CH_2.OH.}\end{array}$$

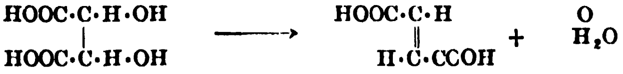
[II]

The change of tartarate into fumarate and alcohol was confirmed by the culture of Rh. Oryzea in the medium consisting of: water 1000 c.c., K_2HPO_4 0.15 g., KH_2PO_4 0.15 g., $MgSO_4$ 0,1 g., $CaCl_2$ 0.1 g., Fe_2Cl_6 and NaCl trace, Na or K-tartarate 20 g., peptone either 1 or 3 gr., or some times 2 g. and $CaCO_3$ 20 g.

Fumaric acid isolated from this culture melted at 279°C (uncor.) in a sealed tube and gave a characteristic colour reaction found by the authors.(5) Its silber salt dried at 110°C was analysed and gave the following data :-

Subst. taken.	AgCl.	Ag as	$C_4H_2O_4Ag_2$
0.2485 g.	0.2242 g.	Found	65.57 %
		Calcul.	65.44 %

Its dimethyl ester melted at 100-101°C. The production of the fumaric acid from tartaric acid may be expressed by the following mode :-



$$\begin{array}{l}\mathrm{HOOC{\cdot}C{\cdot}H{\cdot}OH}\\ \qquad\;|\\ \mathrm{HOOC{\cdot}C{\cdot}H{\cdot}OH}\end{array} \longrightarrow \begin{array}{l}\mathrm{HOOC{\cdot}C{\cdot}H}\\ \quad\;\|\\ \mathrm{H{\cdot}C{\cdot}CCOH}\end{array} + \begin{array}{c}\mathrm{O}\\ \mathrm{H_2O}\end{array}$$

[III]

The formation cf ethyl alcohol by this fungus seems to take place most reasonably in accordance with the equations I and II. If we accept ethes the old theory that lactic acid is an intermediate product of alcoholic fermentation is proved in this case too, as in the case of yeast done by Kayser.(4)

(July 10, 1925).

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(4) Kayser : Comp. R. Tome 176. No. 22. p. 1663. 1923.

(5) Authors method will be published later.

ON THE ENZYMIC ACTIONS OF MALT DIASTASE, PURIFIED FRACTIONALLY BY ETHYL ALCOHOL, UPON POTATO STARCH AND ITS SOLUBLE STARCH.

By Fumiwo Hemmi and Mitsuji Ito.

(Received March. 29th., 1926)

In our previous paper (this Journal, Vol. 1, No. 8), we have reported that the enzymic actions of malt diastase upon potato starch and its soluble starch are different from those upon the other kinds of starch and soluble starch. Since then, we have carried out a further investigation to make the fact more clearly and obtained the following results:

1. Malt diastase saccharifies potato starch more easily than its soluble starch prepared by us according to the Lintner's method.
2. The viscosity of potato starch paste is relatively large, but malt diastase liquefies the paste completely within a short time.
3. For the purification of malt diastase, we extacted malt powder by 20% alcohol (vol.). To the extract, strong alcohol was added making it altogether 55% in volume. The fraction precipitated from 55% alcoholic solution was removed. Then the alcoholic content of the filtrate was increased to 80% by a further addition of strong alcohol. The precipitate of the enzyme thus obtained, and purified again by the repetition of the same process, displayed strong actions of both liquefaction and saccharification upon potato starch paste.
4. Of the fractions of malt diastase purified fractionally by ethyl alcohol, the most active fraction for liquefaction does not coincide with that for saccharification.

(Feb. 28th., 1926.)

RECOVERY AND UTILIZATION OF OLD SLLK DEGUMMING BATHS

By Risaku Tsunokae.

(Received Apr.9th., 1926)

I. In spite of the long history of boiling-off of the silk, it was only towards the last decade of the past century that man felt it mas necessary to recover and reutilize the old silk degumming baths. The much increase in the price of soap, and the difficulty of conducting the boiling-off operation at a sufficiently low cost to compensate for the higher cost of labour, invited man to this consideration.

II. Boiled-off liquor is now employed in the dyeing of silk, as it is found to have good levelling or equalizing properties, by causing the coloring matter to go more slowly on the fibre.

III. The composition of the boiled-off liquor is much liable to variation as the silk scourers differ from one another in the quantity of soap to be used in the treatment of raw silk. The constituents of value in the liquor are, however, no other than the fatty matter and the sericine or silk gum, in every case.

IV. A sample of boiled-off liquor, examined by the author, sho weda specific gravity of 1.010, and contained:

Soap	1.119%
Free fatty acids	0.143
Sericine	0.316

V. Some reagents such as sulphate of alumina, sulphate of copper, lead acetate and chloride of calcium, precipitate both the soap and silk gum as curdy mass. This can be separated through filtration from the quite clear waterly liquid which is in a good condition for running off. The mass of lead soap and sericine thus obtained contains the largest quantity of the silk gum of all, the next is that of aluminium soap, and that of copper soap comes the third.

VI. The mass of copper soap and silk gum, which is precipitated by adding the sulphate of copper into the boiling-off liquor, and separated through filteration, can be elmulsified by the treatment with such emulsifiers as Turkey-red oil, gum arabic, potassium soap etc.. The emulsified copper soap is very good fungicide and has a great demand from gardners and pomologists. The method has been patented for the author. (Japanese Patent No. 65055)

VII. The mass of metallic soap and silk gum thus obtained is mixed with a small quantity of water and strong acid such as hydrochloric acid or sulphuric acid, the mixture of fatty acid and silk gum separates and collects on the surface of the aqueous liquid, and it can be easily skimmed off. The fatty matter can be separated from the sericine by the special steam distillation method, invented by the author, which enables the distillation at by far the lower temperature than the usual method. In this special distillation method, the author uses not only the vapour of the steam, but also utilizes that of the toluene, xylene, or other solvents immiscible to water and having lower boiling points than those of the fatty acids. By this process the distillation temp. of the fatty acid can be depressed about 30—50°C than those of the original ones.

ON THE SCROOP OF THE NATURAL SILK AND THE WAY TO PRODUCE IT ON THE RAYON (ARTIFICIAL SILK)

By Risaku Tsunokae.

(Received March 13th., 1926)

Preface

When the scoured silk fibre is squeezed or pressed it makes a crackling sound which is termed as "Scroop."

Several explanations to account for the scroop have been given, and these can be classified into two groups: the one is that the scroop of the silk is an inherent property of the fibre itself and it is produced by the scouring of it. But if the silk is badly treated in the course of degumming and aftertreatments, the scroop will be lost, but it may acquire again when it is worked in a bath of dilute acids. The other explanation is that the scroop of silk is not the original property of its fibre but an acquired one in the boiling-off operation and other aftertreatments.

On artifcial silks it is generally supposed that the scroop can not be produced on its fibre, but some writer says it can be done by passing the fibre through a soap bath at first and then through a bath containing a small quantity of acid, and drying it without further washing. According to the author's investigations, the crackling sound which can be termed generally as "Scroop" is not the inherent property of the natural silk fibre, but it is a character acquired in the course of scouring and aftertreatments. Moreover, if the conditions which are necessary for producing a "Scroop" on the fibre of natural silk are applied to the fibre of artificial silk, there can be produced on its fibre as good a scroop as that of the fibre of natural silk, and the author's method of producing the scroop on the fibre of artificial silk has been patented for him. (Japanese patent. No. 65671). The same method can be applied on the fibres of spun silk and cotton also.

The author read a paper in the Annual Meeting of the Agricultural Chemical Society of Japan held in April, 1925, on some parts of this investigation, the principal part of which appeared in the Journal of the Dainippon Sanshikaiho (Vol. 34. No. 401. 1925). Adding the recent experimental results, the author read another paper on this investigation in the Meeting of the Agricultural Chemical Society of Japan, held in January 1926. Here the auther wishes to put down the chief points form this last paper.

Experimental.

1. When the natural silks are scoured by means of alkali, or proteolytic ferment (enzyme) snch as pancreatin, or by the hot water under pressure in the autoclave without using marseilles soaps in each case, the "Scroop" is produced to some extent on their fibres, so it can be deduced that the silk fibre has the scroop of some degree as the inherent property of the fibre itself. By treating these scoured silks of different methods of scouring, with diluted acids such as acetic, tartaric etc., the scroops are increased in a degree. But compared with the scroops which is produced on the silk fibre scoured by the soap and after treated with the dilute acid, they are by far the less than those of the latter. In other words, the scoured silk fibre by means of the soap has the peculiar property for producing scroops on its fibre.

2. The scoured silk by means of soap absorbs always fatty acids and soaps on its fibre to the extent of about 1.2% of the weight of the silk. These residual substances in the fibre differ in quantity according to the aftertreatments of scouring process, that is, if the silk is treated by the soda solution, the quantity of the soap increases and that of the fatty acid decreases. On the other hand if the silk is passed through the acid bath at the end of the scouring process, the quantity of the fatty acid increases greatly and makes up more than 95% of the total residual substances. Such silks are always endowed with a good scroop on the fibre.

3. Reversely if the silk, scoured by means of diluted alkali instead of marseilles soap, is treated in the alcoholic solution of fatty acids and next in the diluted acid baths such as acetic, tartaric etc., the scroop can be produced on its fibre as good as that of the silk scoured by the soap. It is evident from these results that the necessary condition for producing the scroop on the silk fibre is the existence of fatty acid in the fibre and the after-treatment of the silk with acid.

4. The scroops produced on the silk fibre by the saturated fatty acids and acetic are rough and greater than those produced by the unsaturated fatty acids and acetic, but the latter is desirable for the natural silk fibre. Among some of the saturated fatty acids, lauric and myristic acids are preferable than stearic and palmitic acids in this respect.

5. A degree of scroop is also greatly influenced by the state of dryness of the material. When the sample is in the perfect dried state after the completion of the scouring operation, it produces the scroop better than that of the less dried samples. But the method of drying, whether it be a steam bath or a vacuum desicator does not matter.

6. There is no relation between the crystalization of fatty acids and their scroop producing power on the fibre.

7. As in the case of the natural silk, the scroop can be produced on the "Rayon" by the treatment with fatty acid solution and the aftertreatment with diluted acids. Unlike that of the natural silk, the saturated fatty acids are better fitted than unsaturated ones in producing scroop on the Rayon (artificial silks).

8. The method of producing "scroop" on the Rayon, which consists in the trearment of the Rayon with fatty acid solution and the aftertreatment with diluted acids, has been patented for the author. But the use of the solution of fatty acids in organic solvents being too expensive, the author recommends now the use of fatty acid as ammonium salts in an aqueous solution.

9. The Rayon thread composed with the fibre of smaller denier, will produce better scroop than that of the greater denier.

10. In order to know the influeuce of the acid used in the aftertreatment upon the fibres of natural and artificial silks, the author examined the stiffness of the fibre by "Serigraph" tests. (Serigraph invented by Dr. K. Hagiwara and K. Tanahashi is used for testing Elasticity of the silk fibre) The Stiffness of the scoured silk fibre decreases by the adsorption of fatty acids in it, while the silk fiber having no fatty acids in it increases its stiffness by the treatment of diluted acid, and the scroop is produced to some extent on the sample. The scoured silk treated with dilute acid in the presence of fatty acids in the fibre, increases its stiffness compared with that of the fibre with fatty acids in it and treated by the acid, but the scroop of the former sample is produced the best of all.

From these experimental results the author concluded that the scroop of the silk fibre is produced by the treatment of it with dilute acid in presence of fatty acids in the fibre.

RESEARCH ON REDUCTION POTENTIALS OF ORGANIC COMPOUNDS

(PART I)

REDUCTION POTENTIAL OF ISOVALERALDEHYE

By Masuzo Shikata and Isamu Tachi

Introduction

Applicability of the dropping mercury cathode for a research on reduction potentials of organic compounds has been proved by one of the present authors by the measurement of R. P. of nitrobenzene.*

The facilities of applying mercury dropping cathode, as it has been already proved, consist in the simplicity of its manipulation and the possibility of measurng minute quantities of reducible substances, for example in case of nitrobenzene the R. P. is determinable in a solution so dilute as to contain 10^{-5}mol of it.

The present authors observed, that some reducible substances were detected in the aqueous solution of Kahlbaum's isoamylalcohol.

The present work, as one of the systematic researches on the reduction potentials of organic compounds, has been undertaken in order to show the possible application of dropping mercury cathode to the qualitative and quantitative analysis of aldehyde.

The possible reducible impurities present in isoamylalcohol are lower aliphatic aldehydes, furfural and pyridine.

In the present report the measurement of R. P. of isoveleraldehyde by the mercury dropping cathode and its relations to the impurities of isoamylalcohol are chiefly described.

Experimental

The experiments have been carried out with the dropping mercury cathode and "Polarograph".×

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*M. Shikata : Trans. Faraday Soc. No. 61 Vol XXI p.42—63 (1925)

×Y. Heyrovský and M. Shikata : Rec. Trav. Chim. des Pays—Bas Tome XLIV(1925)p. 496—498.

The polarograph is a photographic auto-registering apparatus for tracing electrolysing current-voltage curves and has been very much used in the study of deposition potentials of metallic ions to the mercury dropping cathode[**]

For the determination of R. Ps from the current-voltage curve the deposition of turning point of a constant current increment (i. e. 1,9. 10^{-8}amp per 10 mv) has been taken.

The isovaleraldehyde (Kahlbaum) has been purified by the bisulphite method and the fraction between 91° and 92°C has been taken. As the purified and unpurified Kahlbaum's isoveleraldehyde have shown no recognizable difference in the measuremeants, the latter has been used in the most part of those experiments.

In order to remove the atomospheric oxygen, hydrogen gas has been bubbled through the electrolysing vessel. A care has been given to avoid the evaporation of isovaleraldehyde of electrolysing solution, hydrogen gas has been passed through the wash bottle containing the isovaleraldehyde solution of the same composition as the electrolysing solution.

R. P.s have been measured in acidic, neutral as well as alkaline solutions with various concentrations of isovaleraldehyde. Anode potentials (i. e. anode corrections) have been calculated from a normal calomel electrode.

Table I

R. P. s in acidic solutions

Solution	Conc. of isovaler-aldehyde (mol)	Anode potential	R. P. (observed) in volt	R. P. (calc. by formula (2)	π (ob)— π (calc)	R. P. (calc. by formula (3)	π——π (ob) (calc)
0.1n HCl	$1.868.10^{-1}$	+0.051	−1.098	standerd	0.000	standerd	0.000
〃	$9.34\ .10^{-2}$	+0.082	−1.111 (mean)	−1.106	−0.005	−1.104	−0.007
〃	$1.868.10^{-2}$	+0.052	−1.119	−1.127	+0.008	−1.119	0.000
0.01n HCl	$1.868.10^{-1}$	+0.091	−1.125	−1.156	+0.030	−1.127	+0.002
〃	〃 $.10^{-2}$	+0.096	−1.146	−1.185	+0.039	−1.177	+0.029
0.001n HCl + 0.1n KCl	〃 $.10^{-2}$	+0.070	−1.199	−1.243	+0.044	−1.235	+0.036

Table II

R. P. s in neutral solutions

Solution	Conc. of isovaler-aldehyde (mol)	Anode potential in volt	R. P. (observed) in volt	R. P (calc. by formula (2) in volt	π——π (ob) (calc)
3n NaCl	$9.34.10^{-2}$	−0.060	−1.260	−1.441	+0.181
1n 〃	〃	+0.005	−1.261	〃	+0.180
0.1n 〃	〃	+0.042	−1.258	〃	+0.183
3n KCl	〃	−0.065	−1.292	〃	+0.149
1n 〃	〃	−0.003	−1.294	〃	+0.147
0.1n 〃	〃	+0.045	−1.298	〃	+0.143

〃	〃	$1.868.10^{-1}$	+0.066	−1.255	−1.433	+0.178
〃	〃	$1.868.10^{-2}$	+0 064	−1.353	−1.462	+0.109
〃	〃	〃	〃	−1.357	〃	+0.105

Table III

R. P.s in 0.1n NH_4Cl

(mean value of 2—3 times)

Conc. of isovaler aldehyde	Anode potential in volt	R. P. (observed) in volt	R. P. (calc. by formula (2)	π——π (ob) (calc)	R. P. (calc. by formula (3)	π——π (ob) (calc)	Height of saturation curve in Ampere
$1.868.10^{-1}$	+0.050	−1.258	−1.295	+0.037	−1.267	+0.009	——
〃 $.10^{-2}$	+0.059	−1.364	−1.324	−0.040	−1.401	+0.037	——
〃 $.10^{-3}$	+0.058	−1.553	−1.353	−0.200	—1.517	—0.034	$8.1.10^{-6}$
〃 $.10^{-4}$	+0.060	−1.596	−1.382	−0.214	−1.613	+0.017	$7.5.10^{-7}$
$9.34 .10^{-5}$	+0.060	+?	——	——	——	——	——

Further the R. P. s in 0.1 n NH_4Cl solutions with various contents of isovaleraldehyde has been measured to see the shifts of R.·P. s with concentrations of isovaleraldehyde and the form of saturation curves due to isovaleraldehyde.

Table IV

R. P. s in 0.1n NH_4Cl

(mean value of 2 — 3 experiments)

Conc. of isovaler aldehye	Anode potential in volt	R. P. (observed) in volt	R. P. (calc. by formula (2)	π——π (ob) (calc)	R. P. (calc. by formula (3)	π——π (ob) (calc)	Height of saturation curve in Ampere
$1.868.10^{-1}$	+0.050	−1.258	−1.295	+0.037	−1 267	+0.009	——
〃 $.10^{-2}$	+0.059	−1.364	−1.324	—0.040	−1.401	+0.037	——
〃 $.10^{-3}$	+0.058	−1.553	−1.353	−0.200	−1.517	−0.034	$8.1\ 10^{-6}$
〃 $.10^{-4}$	+0.060	−1.596	−1.382	−0.214	−1.613	+0.017	$7.5.10^{-7}$
$9.34.10^{-5}$	+0.060	+ ?	——	——	——	——	——

General Considerations of the Results.

Reduction of isovaleraldehyde can be considered as follows:

$$R.\ CHO + 2H = RCH_2OH$$

If the electrolytic reductions of isovaleraldehyde should proceed in an equilibrium state at the dropping mercury cathodes the R. P.s of isovaler aldehyde solutions should be given by the following formula:

$$\pi = -\frac{RT}{2F} \ln \frac{k'' C_{R.CH_2OH}}{[H\cdot]^2 C_{R.CHO}} \qquad (1)$$

in which $C_{R.CHO}$ denotes the concentration of isovaleraldehyde and $C_{R.CH_2OH}$ that of isoamylalcohol. It has been previously proved that the primary

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**) See for example Rec. Trav. Chim. des Pays—Bas. Tome XLIV(1925) P 499—607.

reduction of nitrobenzene at the mercury cathode proceeds in the equilibrium state.

Moreover, if we determine the reduction potentials of isovaleraldehyde, by taking the same current increment (1,9.10^{-8}amp.), $C_{R.CH_2OH}$ i.e.the concentration of reduced product is constant at the R. P.

So that we obtain for 20°C

$$\pi = -\frac{0.05812}{2} - \log \frac{k}{[H^{\cdot}]^2 C_{R.CHO}} \qquad (2)$$

If we take the R. P. (i. e. at the point of current increment of 1,9 10^{-8} amp) of 0,1n HCl solution, containing 0,1868 gr. mol per liter as standard (R. P. −1,098 volt), we can calculate the value of k

$$\log K = 34,6724$$

In an acidic and alkaline solution the formula (2) holds good to not an unsatisfactory degree; only in a neutral solution R. P. being much positive than expected from formula (2), that is to say, isovaleraldehyde is reduced much easier in a neutral solution than expected from the formula.

If we compare the R. P.s of the same hydroion concentration with various contents of isovaleraldehyde, we can obtain from the shifts of R. P., the coefficient of adsorption isotherm, by the formula proposed by one of us:***

$$\pi = -\frac{RT}{2F} \ln \frac{k}{C_{RCHO}^{\frac{1}{m}}} \qquad (3)$$

For an acidic solution m=1,38, for an alkaline solution m=1.33, revealing some positive absorption of isovaleraldehyde to the mercury cathode in a slightly acidic solution as well as in a neutral solution m is smaller than one. For example in 0,1n NH_4Cl m=0,272, and in 0,1n KCl m=0,287.

It seems to be rather peculiar that m happens to be smaller than one, for such examples have rarely been met with until now. That m is smaller than one means passivity of hydroion discharge of desorption of isovaleraldehyde from mercury surface. This is quite probable, if we remind that nitrobenzene is under ordinary conditions highly absorbed by mercury surface, only when mercury is negatively polarised, the negative charge arising from the negative charge arising from the negative polarisation expels nitrobenzene from the mercury surface, namely the desorption of nitrobenzene occurs.

***) l. c. Trans. Faraday Soc. p52

If such desorption should occur in the case of isovaleraldehyde we can expect that the isovaleraldehyde would not be much influenced by the salting out action of a concentrated neutral solution.

In this respect nitrobenzene under ordinary conditions is much influenced by the salting action of neutral salt, which is revealed by its aquiring much positive reduction potential.

Contrary to nitrobenzene, the isovaleraldehyde solution shows no conspicuous change of R. P. in a highly concentrated neutral salt solution, therefore it seems to be that the desorption must have taken place in this case. Although this is not the only explanation, the present authors are much inclined to take this view.

The height of saturation curve could be applied for the quantitative measurement of isovaleraldehyde. By this means the solubility of isovaleraldehyde in an aqueous solution is estimated to be 0.4475 gr.mol per liter. Further, by this method three reducible substances have been traced in isoamylalcohol of Kahlbaum's preparation. The most probable impurities of isoamylalcohol are aliphatic aldehydes, pyridine and furfural; so the reduction potentials of furfural, pyridine, isovaleraldehyde and isobutylaldehyde have been measured in a neutral as well as in 0.1n NH_4Cl.

Table V

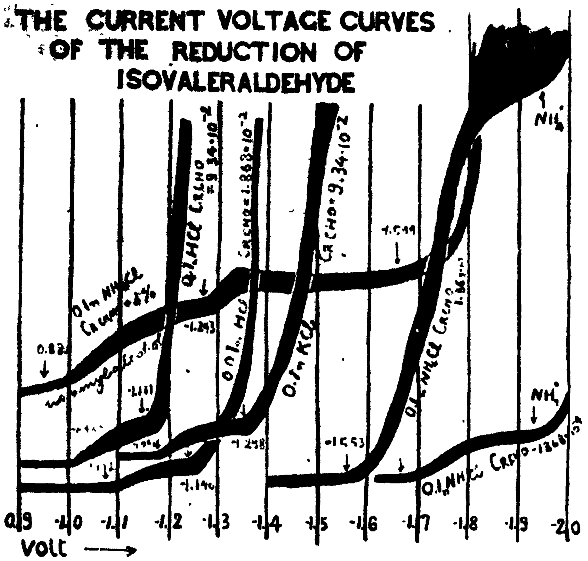
	Concentration	Solution		Reduction Potential			
				I	II	III	IV
Isoamylalcohol (KAHLBAUM) Commercial	3 %	0.1n	NH_4Cl	−0.880	−1.243	−1.599	——
Isoamylalcohol	0.1%	0.1n	KCl	−1.060	−1.263	−1.438	——
〃 (distilled)	1 %	0.1n	NH_4Cl	−0.888	?	−1.559	——
Isovaleraldehyde	$1.868.10^{-3}$m		〃	——	——	−1.553	——
Furfural	0.002%		〃	——	−1.239	——	——
Isobutylaldehyde	1 %		〃	−1.056	——	——	——
Pyridine	0.1251 m		〃	——	——	——	−1 673

It will be seen that R. P. of the second and third reducible impurities coinside with those of furfural and isovaleraldehyde.

In the following diagram, the current voltage curve of isoamylalcohol as well asthose of isovaleraldehyde solutions are shown.

As regards to the Schiff's reagent for the aldehyde test, the limit of detectable concentration is $1.87.10^{-3}$ mol of isovaleraldehyde, whereas by the mercury dropping cathode isovaleraldehyde of $9.34\ 10^{-5}$ mol is detectable i.e. the latter method exceeds twenty times in sensitiveness.

Lastly to give a decisive proof that the reduction product is isoamylalcohol, the influence of isoamylalcohol has been studied and found that the R. P.



of isovaleraldehyde with a definite quantity of isoamylalcohol follows the formula (1) in the first approximation with respect to a system isovaleraldehyde –isoamylalcohol.

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A NEW METHOD OF COLORIMETRIC ESTIMATION OF HISTIDINE

PART I.

By Kôzô Suzuki and Yoshio Kaishio

(Received Apr. 11th., 1926)

(From the Chemical Laboratory of Imperial Zootechnical Experiment Station, Chiba.)

When the histidine solution is added to the solution of diazobenzene sulfonic acid in 10% solution of sodium carbonate, dark red colour is produced (diazo reaction), then the solution is made distinctly acid with strong hydrochloric acid, dark red colour becomes orange, now add zinc dust and allow the reduction to proceed for about 15 minutes. .Render the clear, colourless, supernatant solution alkaline by the addition of strong ammonia. A characteristic golden yellow colouration is produced.

This reaction is known as Totani's reaction for histidine.

We attempted to estimate the amount of histidine colorimetrically by its golden yellow colour of Totani's reaction for histidine and it was ascertained that this reaction can be applied satisfactorily for the estimation of the small quantity of histidine by the following reasons:

1. Totani's reaction produces a characteristic golden yellow colour and the reactian is sensitive.
2. The golden yellow colour of histidine is permanent for a considerable time.
3. Totani's reaction is not affected by the excess of reagents such as sodium carbonate, conc. snlfuric acid, zinc dust and alkalies.
4 The intensity of the golden yellow colour is quite proportional to the amount of histidine.

We have found that a very dilute solution of potassium bichromate is same colour with the golden yellow colour, which is produced by Totani's reaction for histidine and it is convenient to use 1/100 normal solution of potassium bichromate for the standard.

There exists following relation between 1/100 normal solution of potassium bichromate and the amount of histidine:

1 mm. layer of $K_2Cr_2O_7$=0.010932 mg. nitrogen of histidine in 1 cc.

The computation of the unknown amount of histidine can be readily made by means of the following equation:

$$X=\frac{0.010932\times d}{D}$$

X mg. of histidine nitrogen in 1 cc.
D number of mm. of the histidine solution.
d number of mm. of 1/100 normal $K_2Cr_2O_7$ solution.

By the application of this colorimetric estimation method of histidine, hexon bases of the decomposition products of proteins can be calculated asfollows:

Arginine N=4/3 (total base N−amino N−2/3histidine N)
Lysine N=total N−(histidine N+arginine N+cystine N)

ON SULPHUR CONTAINING AMINO-ACIDS. VI.

ON THE PRESENCE OF CYSTEINE GROUP IN PROTEIN MOLECULES.

By Yuzuru Okuda and Yuraku Nishijima.

(Received Apr. 11 th., 1926)

(From the Biochemical Laboratory, Department of Agriculture,
Kyushu Imperial University, Fukuoka.)

The purpose of this investigation was to make some quantitative studies concerning the presence of cysteine group in the protein molecule, a subject on which, as far as we know, no work has hitherto been done.

The summary and conclusion of the investigation were as follows:

(1). The authors have failed to confirm the findings of Mörner that cysteine is produced secondarily from cystine during prolonged hydrolysis of proteins with hydrochloric acid.

(2). The authors have demonstrated that the procedure of Mörner for the identification of cysteine in a protein-hydrolysate is erroneous, and have verified the fact that cysteine is not produced from cystine, but that on the contrary cysteine, in the course of hydrolysis, gives rise to cystine, and that both cysteine and cystine are the primary products of the complete hydrolysis.

(3). In accordance with the results of investigation of Heffter and Arnold, the authors have found that some proteins give the nitroprusside reaction while others do not, and came to the conclusion that there are sulphur-linkages, such as R–SH, R–S–R and R–S–S–R, in protein molecules. The protein containing R–SH group gives the reaction directly, the one containing R–S–R group indirectly or after a certain degree of hydrolysis, and the other containing R–S–R alone does not.

(4). Cysteine and cystine were determined, by means of the iodine-method, in the several stages of hydrolysis of proteins. Egg albumine and wool gave only a minute quantity of cysteine in comparison with that of cystine but muscle-proteins freshly prepard gave much cysteine, for instance, the muscle protein of Pagrus, when hydrolysed with hydrochloric acid in the current of carbon dioxide gas, gave nearly an equal amount of cysteine and cystine. When it is borne in mind that cysteine is easily oxidizable to cystine, during the preparation of proteins, and especially in the instant of the cleavage of proteins, the cysteine content of living muscle proteins should be predominate to their cystine content.

(5). The effect of acid hydrolysis upon cysteine was studied with pure cysteine and with a mixture of cysteine and gelatine which contained no cysteine. The hydrolysis was performed in the usual way or in the air. The quantity of preformed cysteine and cystine produced were determined quattitatively. The oxidation was slow and the cysteine reaction disappeared after about 100 hours of hydrolysis but when muscle proteins containing the cysteine group was treated in the same way the oxidation was more rapid, and after 20 hours the cysteine reaction was negative. From this experimental result we see that isolated cysteine is more stable than cysteine in the nascent state, which is reactive and autoxidizable.

(6). When cysteine was boiled with hydrochloric acid as mentioned above it became cystine quantitatively.

ON THE HORDEIN OF THE NAKED BARLEY.

By Eiji Takahashi and Kiyoshi Shirahama.

(From the Food Laboratory, College of Agricurture, Imperial University of Hokkaido.)

(Received March 23rd., 1926)

Three Kinds of Alcohol soluble proteins were separated by the authors from new and old naked barley(Hordeum Coeleste L.)according to Osborne's method.

(1) The old barley flour was extracted with 70% cold alcohol. The resulted solution was condensed to a small volume and cooled, when a jelly like substance was deposited.

Dissolving it in a little quantity of dilute alcohol, pouring into the cold water (0—5°), the protein was again deposited.

After this treatment was repeated three times it was redissolved in a small quantity of dilute alcohol when this solution was the absolute alcohol, a firm coagulum (A) was formed in the milky solution. By condencing the mother solution separated from (A) to a small volume and pouring into the cold water, it produced the second yield (B).

(2) From the new barley flour we obtained by the same method also two proteins (C) and (D).

But (B) and (D) were proved afterwards to be identical.

We studied these three proteins on their physicochemical and chemical properties and found the marked differences among them in the following points.

(1) The color reaction.

(2) The behaviours for water and alcohol.
(3) The turbidity in relation to concentration of alcohol and temperature.
(4) The surface tension of alkaline solution of these proteins.
(5) The elemental composition.
(6) The proportion of nitrogen.
(7) The ratio of total sulphur to sulphide sulphur.

From above results, we found that the alcohol soluble protein of naked barley was distinctly different from hordein which was described by Osborne in 1895 and by others,

	Hordein of Common Barley.	Hordein of Naked Barley.
(1)	Homogenous substance	Not homogenous, but probably mixture in most occasion.
(2)	With sulphuric acid it gives red color.	Purple red–red

(3) Elemental compositon

		C	A	B
C	54.29	56.16	55.97	54.72
H	6.80	6.65	6.64	6.50
N	18.21	17.15	16.36	15.18
S	0.83	0.19	0.33	0.63
O	20.87	19.85	20.70	22.97

(4) Distribution of nitrogen.

			C	A	B
Total	N	17.21	17.15	16.36	15.18
Amide	N	4.10	4.00	3.03	2.00
Humine	N	0.23	0.31	0.22	0.22
Diamino	N	0·77	3.01	2.70	2.21
Mono-amino	NN	12.20	9.83	10.41	10.72

Percentage for total nitrogen.

			C	A	B
Total	N	100.00	100.00	100.00	100 00
Amide	N	23.00	23.32	18.52	13.17
Humine	N	1.70	1.81	1.34	1.45
Diamino	N	7.69	17.56	16.50	14.75
Mono-amino	N	67.61	57.31	63.64	70.63

But a further research will be required to establish the difference more clearly.

NOTE ON THE EXTRACTION OF SILK-FIBROIN BY FORMIC ACID.

By Tokuhei Kametaka.

(Received May 24 th., 1926)

I. Extraction and Purification.

Silk-fibroin is somewhat soluble in pure formic acid, but it is not so easily soluble as is given in many literatures (Baumann u. Diesser, C. 1911, I, 442 among others). This may be clearly seen from the following example of the extraction of fibroin by formic acid. 4.8g. of silk-fibroin and 50g. of pure formic acid, Kahlbaum, were heated together in a flask with reversed condenser in an oil bath of about 110° for 3 hours. Fibres gelatinized and partly dissolved. The solution was filtered from undissolved residue by water pump, and then the formic acid was distilled off almost completely under reduced pressure at about 60°. To a little brown residue absolute alcohol was added and rubbed with glass rod; white amorphous substance separated, which was filtered and dried in a vacuum desiccator. This first extract weighed 2.2g, or 44% of the original fibroin.

Insoluble residue after first extraction was similarly extracted with 25g. formic acid, and the second extract was 1.3g. or 27%. Similarly, the third extract was 0.9g. or 19%, and the residue was 0.3g. or 6 %. Thus even after three extractions still 6 % insoluble residue remains.

These extracts, when treated with hot water, separate into two parts, one soluble and the other insoluble in water. The solution was decolorized with animal charcoal, in which about half the substance is adsorped and lost, and the solution was evaporated on water bath almost to dryness and separated by adding absolute alcohol. This is the only method of purification.

II. Properties and Molecular weight of the Soluble Part.

It is white amorphous powder, and is almost all soluble in hot-water, but a small quantity of flocculent insoluble particles, into which the soluble part seems to transform on standing, always remains. The solution, when saturated with ammonium sulphate, precipitates casein-like substance.

Heated in a capillary tube, it blackens at about 200°, and decomposes at about 240°.

The substance contains very little free amino-nitrogen, 0.62% as determined by formol-titration; but after hydrosis by boiling with alkali and then neutralizing, free amino-nitrogen increased to 8 %.

Following Herzog and Kobel,(1) molecular weight determinations by freezing method, resorcin as solvent, were tried, with following results:-

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(1) K. O. Herzog und M. Kobel, Ztschr. f. physiol. Chem. 134 296—299, 1924.

Silk-fibroin	210 (Herzog and Kobel found 200),	
Extract by formic acid, Insoluble Part		240,
〃 〃 〃 〃, Soluble Part		253.

While association-products of two molecules of anhydride of dipeptide from glycine and alanine, the substance assumed by Herzog[2] as the main constituent of fibroin, is $(C_5H_8N_2O_2)_2=256$.

Owing to the lack of material, the constituent amino-acids of the soluble part could not be determined by hydrolysis. But the substance showed very faint Millon's reaction. So probably it contains no tyrosine, and may be composed only of glycine and alanine.

III. Elementary Analysis of Soluble Part.

As the substance was heated with animal charcoal, dissolving out its mineral contents, and finally precipitated by absolute alcohol, it contains more ash (3–4%) than fibroin itself, and in spite of much effort ash-free substance conld not be obtained.

Four analysis gave following average values (ash being diminished).

C 41.30, H 6.91, N 18.48

while fibroin itself gave

C 48.74, H 6.42, N 18.86

Too low value of carbon for soluble part is noticeable.

THE INVESTIGATION OF FOOD AT THE SPINNING-FACTORIES.

By Masao Shimidzu.

(From The Municipal Hygienic Labolatory
of Osaka, Japan.)

(Received Feb. 10th., 1926)

Besides the theoretical studies on nutrition, it is evident that the investigation of foodstuffs actually taken by the population is also important. The author has taken up the investigation of the diet in various classes. Here will be reported the results of the author's investigations among the factory girls of three large spinning factories at Osaka.

Period of investigation.	one month each.
Average weight of body.	43.8 kg.
Average age.	18.
Total number of girls examined.	7052.

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(2) Foot note of above literature.

The amount of nutrients and calories taken per day per capita is as follows:

protein(g)	fat(g)	carbohydrate(g)	Calories.
71.5	13.4	467.9	2336
13.0%	2.4%	84.6%	Total 100%

Classifying the proteins according to their respective origins, their percentages are as follows:

Total protein	100		
Vegetable origin	82. %	Animal origin	18. %
from rice	41. %	from beef & egg	2.2%
from barley	6.3%	from fish, etc.	15.8%
from beans	26.7%		
from vegetables	8.0%		

The amount of inorganic salts (especially CaO, MgO,) were calculated, from the kown data, but some of the particular food-stuffs were put into chemical analysis. It was found that each girl takes in, in average, 0.666g CaO and 1.154g MgO per day.

The author fed albino rats on the diet of the same ratio as that of the factories. The basal diet contained about ten different sorts of foodstuffs and the author added to it various nutrients, calcium, vitamins, various proteins, etc. to examine what kinds of nutrients are deficient.

According to the results of animal experiments, the diet seems to be sufficient in all respects, except in vitamin B, for the diet neither kept the body weight of the rats under examination, nor indicated any curative effect on the troubles caused by the deficiency in vitamin B.

The diet may therefore perhaps be deficient in vitamin B in the case of the factory girls for their average age.

ON THE COLLOIDAL MAGNESIUM SILICATE

By Hideo Kaneko.

(Received March 20th., 1926)

The author prepared magnesium silicate colloid by the interaction of sodium silicates (Na_2O,SiO_2, $Na_2O,2SiO_2$, $Na_2O.3SiO_2$, $Na_2O.4SiO_2$) prepared by the Asahi Glass Company and magnesium sulfate. Like plant oxidase, magnesium silicate colloid oxidises aromatic substances containing an ortho-dihydroxy grouping such as catechol, pyrogallol, and myricetin. This is expressed as a unimolecular reaction.

$$K = \frac{2.3}{\beta t}\left(\beta \log \frac{\varepsilon\infty}{\varepsilon\infty - \varepsilon} + \varepsilon\right)$$

ε : Extinction coefficient (measured by Natting's spectrophotometer)

ε_∞: Extinction coefficient at infinite time.

$\beta = -\frac{1000}{n}$, n : number of time in hour

K : reaction velocity constant.

t	A (Angle of rotation)	ε	K
0	56.5	—	—
1	59.0	0.00610	0.001452
2	61.0	0.01126	1403
3	62.7	0.01608	1355
4	64.6	2190	1430
5	66.1	2686	1437
6	67.4	3144	1414
24	76.0	7164	—
		mean	0.001430

Tannin solution turns rapidly brownish black in the presence of amino acid or its salt, when added with the magnesium silicate colloid. The hydrolysis of starch solution by acid is also stimulated by it.

The colloid which is buried in the soil shows a little stronger oxidising action than the one exposed to the sunbeam. The viscosity of the colloid is little influenced by the addition of potassium salt and the latter is also most absorbed of all alkali salts by the former.

Magnesium silicate colloid having the concentration between $\frac{1}{1000}$g. and $\frac{1}{2000}$g. protects to some degree the precipitation of calcium carbonate and silver chromate from their solutions.

ON THE PRODUCTION OF AMINES BY ASPERGILLUS ORYZAE.

By Masakazu Yamada and Shō Ishida.

(Received June 2nd., 1926)

The production of diamines, such as putrescine and cadaverine, have hitherto been observed in the bacterial decomposition of proteins (Ellinger, Z. physiol. Chem. **29**, 334, 1900), in the autolysis of yeast (Schenck; Wochsch f. Brau. **22**, 221–27, 1905; K. Kurono; Journ. Chem. Soc. of Japan **36**, 1127-52, 1917) and in the ergot by the action of higher fungus. (Rieländer; Sitzungber. Gesellsch. Naturw. Marburg. 5, Aug. No. 7, 1908).

Lately one of the authors contrived a very suitable method for the isolation of diamines with naphthol yellow and actually separated cadaverine in every case and putrescine attaines from the Japanese brewery products saké, shōyu, miso and nattō. (M. Yamada; this Journ. Vol 2, 39–41. 1926).

It is well known that these products except the case of nattō are brewed by the cooperation of several bacteria, yeasts and moulds so that as the agent of amino-formation the action of moulds, especially of Aspergillus sp., ought not to be overlooked. The part played by the mould is the most important. It is used ordinally at the first stage of the various brewings in Japan, in the form of "koji".(1)

We used soy-bean as the source of protein and made Asp. oryzae No.54. develop on it in a wholly pure state. After about 16 days' culture, 1kg. of koji (water; 57.41%) was used for the isolation of amines by means of naphthol yellow method. By this process, putrescine and cadaverine were separated as their picrates,(2) each weighing 0.463g. and 0.483g, along with a large quantity of ammonia.

It can be concluded that same higher fungi also produce amines from proteins.

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(1) Kōji is prepared from the steamed rice in case of saké, steamed mixture of wheat and soy-bean in case of shōyu, steamed soy-bean only in case of tamari-shōyu or steamed soy-bean with or without the addtion of rice or wheat, according to the kinds, in case of miso, upon which the mycelium of Aspergillus oryzae has been developped in every case.

(2) They were identified in each case from their melting point and also from the content of picric acid by means of Bussh's nitron reagent.

STUDIES ON SOIL PROTOZOA.

I. Influence of soil protozoa on nitrogen fixation of Azotobacter.

Keizô Hirai and Iwao Hino.

(Received June 5th., 1926)

The authors present in this paper their experiments carried out in order to ascertain the facts previously shown by Nasir on the influence of soil protozoa upon nitrogen fixation of Azotobacter.

The authors found that the nitrogen fixation of Azotobacter is generally stimulated and not inhibited in the presence of soil protozoa.

In the presence of soil protozoa, the highest fixation of nitrogen is recorded in this experiments to be 37.70% over the control plot in a sand culture. Out of fifteen experiments eleven showed a decided gain in nitrogen fiixation over the control and two showed no gain, while two gave negative results which are only the cases when the saturated sandy soil is used.

pH value of the nutrient media always changes in the course of experiments: when azotobacter alone exist the medium considerably acidifies, and when soil protozoa coexist it slightly acidifies, while in the single existence of soil protozoa it alkalifies in all cases.

It seems to the authors that the soil protozoa and azotobacter live in the state of disjunctive symbiosis or of metabiosis in the strict sense: in other words, the presence of soil protozoa decreases the acidity of the nutrient media, resulting the vigor of growth and increased fixation of azotobacter, consequently giving favorable effect on the protozoa themselves to increase the vigorosity and multiplication, thereby keeping the active state of protozoa for a longer period.

ON THE SOME NITROGENOUS CONSTITUENTS OF THE LEAVES OF KUZU (Preliminary report)

(The Japanese arrow-root plant, Pueraria hirsuta, Matsum.)

by Rinjiro Sasaki

(Received June 17th., 1926)

Many vegetables contain less nitrogen than grains and even if, sometimes, comparatively much nitrogen is contained in a vegetable, the great part of it is of non-protein nature. But there are some wild vegetables that contain comparatively much protein nitrogen. At present, the nutritive value of vegetables is thought to be depend on their mineral matters (ash) and various kinds of vitamins, and the value of the nitrogenous material is almost neglected.

Various green forage plants contribute appreciable amounts of protein to the ration of farm animals, but practically nothing is known of the chemistry of these, and even the proportion of protein in these plants is not yet established.

In our laboratory the white rats were fed on polished white rice or barley, each added only fresh vegetables, without other supplements. These rats maintained normal growth and nutrition and in some of them two generations succeeded. It is the known fact that the white rat fed exclusively on polished rice can not grow normal for the deficiency of ash and vitamins and also probably of protein. The author thinks, the above mentioned normal growth is resulted from the supply of good nitrogenous materials, as well as of the mineral matters and vitamins from vegetables.

A serious gap exist in our current knowledge of the chemistry of nutrition which makes it impossible to apply to the practical problems of feeding on the farm what has been learned of the nutritive value of the proteins of the cereals as well as of the protein concentrated.

There are not so many investigations referring to the nitrogenous constituents of green forage.

The Japanese arrow-root plant *KUZU* (Pueraria hirsuta, Matsum.) are a leguminous plant and grow wild everywhere in Japan. The leaves contain fairly much nitrogen and are used as fodder. In preparing the hay, the leaves are easy of drying in the air and difficult of dropping from the stem. When it is mixed to the other fodders, it stimulates appetite, so it is usually thought as having special action for maintaining of health of farm animals.

The investigation reported preliminary in this paper embodies attempts to examine the nutritive value of vegetables and also the chemical properties of the protein of the arrow-root leaves.

The wild green arrow-root plant was harvested at the time of full bloom and dried up in the direct sunlight and preserved.

(I) General composition

The general composition was examined by the usual method.

Table I

General composition of arrow-root leaves

Water (%)	Dry matter (%)	In dry matter (%) Crude Protein	True Protein	Ether Extract	Crude Fiber	Crude Ash	Nitrogen-free Extract
10.34	89.658	18.148	17.262	4.799	24.915	8.278	43.860

In 100 Parts of Crude Ash (%)

SiO_2	SO_2	P_2O_5	Mn_3O_4	CaO	MgO	Fe_2O_3	Al_2O_3	Na_2O	K_2O	Undetermined	Cl
11.080	1.935	5.086	0.583	39.071	6.278	0.790	1.340	11.002	20.954	0.873	1.302

(II) Solvents for extraction of nitrogenous constituents.

As it will beseen from Table I, the leaves of arrow-root plant contain much protein. Naturally we must employ the best solvent or apply the best treatment for the isolation of the protein. The author examined various solvents to select the most suitable one for the extraction of nitrogenous matter. 250 ccm. of each of these solvents were applied to the different lot of 5 grm. of the same sample and the extraction durated for 24 hours. In the case of the alkaline alcohol solvent, it was boiled for five minutes with shaking from time to time, and the total nitrogen was estimated in 100 ccm. of liquid. The results are shown in Table II.

Table II

The amounts of nitrogen extracted by various solvents.

	In dry matter (%)	In total N (%)
Total N	2,904	——

Protein N	2.762	95.117
Non-protein N	0.142	4.883
Water soluble N	0.375	12.915
10% NaCl 〃 N	0.368	12.684
5 % CH_3COONa 〃 N	0.454	15.646
0.2 % NaOH 〃 N	0.689	23.742
75 % alcohol 〃 N	0.134	4.608
60 % alcohol containing 0.3 % NaOH soluble N	1.732	59.689
Ether soluble N	0.015	0.526
Water soluble N in the residue of ether extraction	0.343	11.826
60 % alcohol containing NaOH soluble N in the residue of ether and water extraction	0.305	10.515

(III) Extraction and preparation of nitrogenous constituents from the leaves.

The powdered leaves of the arrow-root plant (500 grms.) were extracted by boiling with 60 per cent alcohol containing 0.3 per cent NaOH. The liquid was separated by the filter cloth. The resulting extract was filtered through paper pulp, which removed a small amount of soil material and the green colloidal substance. The filtrate was neutralized by the addition of dilute HCl, but there was no appreciable precipitate. Then this solution was concentrated to about 2000 ccm. in vacuo. To this concentrated solution which contained a small amount of precipitate was added the requisite amount of HCl to effect a complete precipitation. The precipitate was amorphous and was insoluble in excess of HCl. This was separated by centrifuging. It was redissolved by adding dilute NaOH and filtered through paper pulp, and the clear but brown colored solution was obtained. The addition of the requisite amount of HCl to the filtrate caused the precipitate to separate. Thus purified, the precipitate was centrifuged off, washed with distilled water acidified with small amount of HCl, and dried in the air. The weight, moisture-free, was 30 grms. and the yield of nitrogenous matter was 34 per cent of the total protein nitrogen.

(IV) Chemical properties.

This is insoluble in either dilute or strong HCl, but soluble in small excess of NaOH to give a clear yellowish brown solution. This is insoluble in ether and alcohol. The precipitate is produced on adding dilute acetic acid to the alkaline solution and it dissolves to an opalescent solution in excess of acetic acid, and the precipitate is not produced on dilution. When calcium chloride solution is added to an alkaline solution, a brown precipitate is formed. The mixture of glacial acetic and conc. HCl dissolves completely the precipitate, but on dilution with water the precipitate is again produced.

Millon's, Adamkiewicz's, Molish's reaction and Xanthoproteic reaction did not distinctly appeared by the abstraction of pigment. The Ninhydrin reaction appeared clearly in the acid-hydrolyzed solution.

(V) Analysis of the precipitate.

The moisture-free precipitate contains 14.153 per cent of nitrogen, 0.58 per cent of ash and some of phosphorus. The nitrogen content, ash-free, is 14.236 per cent. Molish's and furfurol reaction were positive, showing the presence of carbohydrate in the preparation, but the author have not, as yet, had an opportunity to determine whether it is conjugated or impurity. Table III gives the distribution of nitrogen by van Slyke's method after hydrolysis for 48 hours with 20 per cent HCl.

Table III

The distribution of nitrogen

Total nitrogen used for analysis was 0.3500 grm.

	Nitrogen (grm.)	In total N (%)
Amide N	0.0144	4.11
Humine N	0.0969	27.69
Total N in Phosphotungstate Precipitate	0.0545	15.57
Amino N 〃 〃 〃	0.0206	5.89
Arginine N	0.0308	8.80
Cystine N	0.0005	0.14
Histidine N*	0.0162	4.63
Lysine N	0.0070	2.00
Total N in Filtrate (Mono-amino acid)	0.1685	48.14
Amino N in Filtrate	0.1219	34.83
Total N recovered×	0.3343	95.51
Total N of Monoamino-dicarboxylic acid (Total N used for analysis was 0.3212 grms.)	0.0866	2696

*The formula is: Histidine N = 1.5 Total non-amino N − 1.125 Arginine N.

×Andersen-Müller's procedure was adopted.

(VI) An unknown crystalline nitrogenous substance.

The filtrate obtained from the precipitate produced by adding dilute HCl to the concentrated extract of the sample (500 grms. of leaves), was concentrated to about 2000 cc. in vacuo and was filtered again through a paper pulp.

The transparent dark brown filtrate thus obtained was allowed to settle in the cold. The crystalline precipitate was produced. This was separated by centrifuging from mother liquid, washed with the mixture of absolute alcohol and ether, and dried. The precipitate purified in this way represents light greenish tetragonal prisms, melting at 185—187°C, and was completely soluble in NaOH, giving a deep yellow brown solution. The crystal is soluble in acetone and dilute alcohol but not in ether and absolute alcohol. In an acetone solution it is precipitated by the addition of NaOH. In an alkaline solution it is precipitated by the addition of $CuSO_4$. Millon's and Xanthoproteic reaction are positive.

ON THE COMBUSTION TEMPERATURE OF CIGARS AND CIGARETTES.

By Masuzo Shikata.

(Received Apr. 11th., 1926)

It is rather noticeable, that, although so many analysis have been carried out as to the chemical composition of smoke of cigar, no attempt seems to have been undertaken for a measurement of actual combustion temperature of cigars and cigarettes.

The present author has measured the temperature of combustion with a small enamelled iron-constantan thermocouple with its diameter of 0.14 mm.

This thermocouple, when inserted directly in the axial center of cigars and cigarettes can give actual temperature of such small body as cigarette, owing to its very small heat capacity. The calibration of this thermocouple has been done with melting points of metallic tin, zinc and antimony.

The supply of air, of course, is one of the most important factors of combustion, that is to say, smoking, or better to say, sucking of cigarettes is one of the decisive factors for the combustion temperature.

Special cares having been payed in this respect, the measurements have been carried out.

The following table shows the stable maximum temperatures, i. e. maxima of stable temperature without sucking or with very slow sucking.

Table

Numbers of experiments	Asahi	Shikishima	Golden Bat	Star	Westminster	Cigar
1	515	733	649	587	649	597
2	473	671	583	420	753	469
3	552	609	557	649	743	671
4	463	597	597	701	738	629
5	582	582	672	567	701	687
6	530	577	493	592	733	
7	619	520	649	671	749	
8	597	567	602	619	748	
9	711	557	530	723	671	
10	614	676	733	691	795	
Mean	565.8	608.9	606.4	622	726.5	646.6

In the table, the first line is the names of cigars and cigarettes.

Asahi, Shikishima, Golden Bat and Star are Japanese cigarettes prepared by the Japanese Tobacco Monopoly Bureau. The cigar is "Orientales", also from the Monopoly Bureau. Westminster is Turkish Blend A. A. Grade, prepared by Westminster Tobacco Co. Ltd. London.

In Fig. 1. (in the original paper) the method of measurement has been graphically shown. In Fig. 2. the temperature change with time is manifested. Temperature is given by ordinate, time by abscissa in minutes. In this diagram process of combustion are clearly seen.

In case of the cigar the temperature increases gradually.

Such condition must be favourable for the distillation of nicotin, nicotein and other essential components.

In case of cigarettes the maximum temperature is attained suddenly, so that heat decomposition must set in, before most parts of essential components can be distilled or sublimated. It can be noticed that cigarettes of Westminster Tobacco Co. shows much higher combustion temperature than ordinary cigar, and latter has again higher combustion temperature than the Japanese cigarettes.

ON THE PRESENCE OF CYSTEINE GROUP IN PROTEIN MOLECULES.

By Yuzuru Okuda.

(Received May 29th., 1926)

The purpose of this investigation was to make some quantitative studies concerning the presence of cysteine group in the protein molecule, a subject on which, as far as we know, no work has hitherto been done.

I. Is Cysteine Produced Secondarily from Cystine during Prolonged Hydrolysis of Proteins?

Mörner(3) has hydrolyzed some horn substance with hydrochloric acid for a week, and after removing the most part of the acid by evaporation, neutralized the hydrolysate with lead oxide, treated with hydrogen sulphide, and then filtered the precipitate, and after perfectly removing the excess of hydrogen sulphide tested cysteine in the filtrate by means of the nitroprusside reaction. And he has stated that cysteine is produced secondarily from cystine during prolonged hydrolysis of the protein. But we have failed to confirm his conclusion. We have repeated his procedure with pure cystine and with some keratine-cleavage-products free from cysteine, and have verified the facts that by Mörner's process cysteine should be produced from cystine by the reducing action of hydrogen sulphide, but that cysteine is not produced from cystine during prolonged hydrolysis and on the contrary cysteine, in the course of hydrolysis, gives rise to cystine, and that both cysteine and cystine are the primary products of the complete hydrolysis.

II. The Qualitative Test of Cysteine in Proteins.

Heffter[3] and Arnold[1] have demonstrated that some proteins give the nitroprusside reaction while others do not. We repeated their experiments with various proteins, directly or after the treatment with enzymes and oxidizing agents, and have confirmed their findings, and came to the conclusion that there are sulphur linkages, such as R–SH, R–S–R and R–S–S–R, in protein molecules. The protein containing R–SH group gives the reaction directly, the one containing R–S–R group indirectly or after a certain degree of hydrolysis, and the other containing R–S–S–R alone does not.

III. Presence of Cysteine in the Protein-hydrolysates.

For the purpose to show the presence of cysteine in the hydrolysate of proteins, cysteine and cystine were determined, by means of the iodine-method[4], in the several stages of hydrolysis of proteins. Egg albumine and wool gave only a minute quantity of cysteine in comparison with that of cystine but muscle-proteins freshly prepared gave much cysteine, for instance, the muscle-protein of pagrus major, when hydrolyzed with hydrochloric acid in the current of carbon dioxide gas, gave nearly an equal amount of cysteine and cystine as shown in the following table.

Hour of hydrolysis	Cysteine	Cystine	Ratio		Cysteine-reaction	Remarks
			Cysteine	Cystine		
3	33.8	36.1	94	100	+	Hydrolyzed and treated in CO_2 gas.
5	34.1	36.4	94	100	+	
7	34.8	37.4	93	100	+	
20	0.0	98.4	0	100	–	In the air.

When it is borne in mind that cysteine is easily oxidizable to cystine, during the preparation of proteins, and especially in the instant of the cleavage of protein molecules, the cysteine content of living muscle-proteins should be predominate to their cystine content.

IV. The Effect of Acid Hydrolysis upon Cysteine.

For the purpose to know the rate of oxidation of cysteine to cystine, the effect of acid hydrolysis upon cysteine was studied with pure cysteine and with a mixture of cysteine and gelatine which contained no cysteine. The hydrolysis was performed in the usual way or in the air. The quantity of performed cysteine and cystine produced were determined quantitatively. The result of an experiment performed with a mixture of cysteine and gelatine was as follows:—

Hours of hydrolysis	Cysteine found	Cystine found	Rate of oxidation (%)
2	0.90	0.21	18.6
4	0.86	0.24	22.1
6	0.78	0.33	30.0
8	0.73	0.38	33.8
10	0.67	0.44	39.4
15	0.65	0.47	41.5
25	0.60	0.51	45.5
30	0.56	0.55	49.3
54	0.36	0.75	67.1
82	0.14	0.95	87.8
100	0.00	1.01	100.0

In this case, the oxidation was slow and the cysteine reaction disappeared after about 100 hours of hydrolysis, but when muscle-proteins containing the cysteine group was treated in the same way the oxidation was more rapid, and after 20 hours the cysteine reaction was negative. From these experimental results we see that isolated cysteine is more stable than cysteine in the nascent state which is reactive and is easily oxidizable to cystine in the instant of the cleavage and during the hydrolysis, and that when cysteine was boiled with hydrochloric acid as mentioned above it became cystine quantitatively.

Conclusion.

So far as these experimental results are concerned, we came to the following conclusions:— During prolonged hydrolysis of proteins, cysteine is not produced from cystine but on the contrary cysteine gives rise to cystine. A cysteine group is present in some protein molecules and cysteine found in the hydrolysates. Both cysteine and cystine are the primary products of the complete hydrolysis.

My thanks are due to Mr. Y. Nishijima, my assistant, for his analytical work.

Reference.

(1) Arnold: Zeitschr. f. physiol. Chem., **70**, 300, 1910—11.
(2) Heffter: Jahrber. ü. d. F. d. Tierchem. **37**, 565.
(3) Mörner: Zeitschr. f. physiol. Chem., **28**, 595, 1899.
(4) Okuda: J. Departm. of. Agr. Kyushu Imp. Univ., **1**, 163, 1925.

SUR LES PRODUITS DE LA FERMENTATION DU MONASCUS PURPUREUS (CHAMPIGNON DE L'ANG-QUAC).

Par Yusuke Sumiki.
(Collège de chimie agricole de l'Université de Tokyo).

(Received June 5th, 1926)

Le genre Monascus a été classifié en deux espèces, cést-à-dire Mon. ruber et Mon. mucoroides par Van Tieghem. (Bull. de Soc. Bot. de France,

31,1884.)

F. A. Went a isolé une nouvelle espèce du champignon de l'ang-quac et a donné à cause de sa couleur pourpre le nom Mon. purpureus. Il a étudié sa morphorogie, son développement et sa nourriture. (Ann. Soc. Nat. Bot., 8, 1, 1895.)

Prinsen Geerlig a fait une étude des propriétés chimiques de matière colorante de l'ang-quac. (Chem. Ztg., 19,1311, 1895.)

Ueda a prouvé l'existence du Mon. purpureus dans "Beni-koji" de l'Anchû. (Tokyo Bot. Mag., 1902,)

S. Ikeno (Ber. Deut. Bot. Ges., 11,259,1903.) et H. Kuyper (Ann. Myco., 3,32,1905.) ont étudié sur les divers points le développement de périthèces et la formation de spores.

Récemment S. Hagiwara a étudié les enzymes et la matière colorante de ce champignon. (Taiwan Kôgyôbu Hôkokusho, 5,1924.)

EXPERIENCE. I. Les produits de la fermentation.

J'ai cultivé le Mon. purpureus à la température de 26—30° pendant 14—50 jours dans l'extrait kojique ou dans une solution nutritive suivante:

Glucose ou sucre de canne	10g.	KH_2PO_4	0.015g.
Peptone ou KNO_3	0.1—1g.	K_2HPO_4	0.015g.
$MgSO_4$	0.01g.	$CaCl_2$	0.010g.
$FeCl_3$ et NaCl	trace.	Eau distillée	100g.

On distille la solution cultivante de ce champignon à la vapeur d'eau. Après l'évaporation du résidu, on l'acidifie et l'extrait avec l'éther. On obtient l'acide succinique et l'acide lactique de cette solution éthérée (A).

On ajoute l'excès du carbonate de barium dans ce distillatum à la vapeur d'eau et le chauffe dans le ballon fourni d'un réfrigérant descendant, on filtre le contenu du ballon et distillele filtratum à la vapeur d'eau encore une fois. On obtient un distillatum(B) contenant de l'alcool éthylique, de l'aldéhyde acétique et de l'huile de fusel.

Après l'évaporation du résidu de la distillation à la vapeur d'eau, on obtient des sels de barium des acides volatils (C); acides formique et acétique.

Présence de l'alcool éthylique.

Par la répétition des distillations fractionnées du distillatum (B), on obtient une fraction 78°, l'on ajoute l'oxyde de calcium pour échapper de l'eau. On chauffe avec la quantité théorique du isocynate de phenyl au bain-marie dans le tube fermé. On obtient des crystaux longues aiguilles de phenyl uréthane fondant à 50—52°.

0.0924g. Subst. ont donné 7.1cm^3 N_2 (24°, 758m.m.).
Calculé pour $C_9H_{11}O_2N$ N 8.49%.
Trouvé N 8.55%.

Présence de l'huile de fusel.

On trouve la présence de l'huile de fusel dans le distillatum (B) par les réactions suivantes:

Réaction de T. Takahashi (Ztschr. f. N. u. G., 27,820,1914.).

Réaction de Komoroski (Chem. Ztg., 27,808,1903.).

Réaction de Udrannszky (Ztschr. f. Phy. Chem., 13,261,1888.).

Présence de l'aldéhyde acétique.

Par la distillation fraetionnée du distillatum(B), on obtient une fraction 50—78° qui donne une couleur rouge avec le réactif de Schiff.

Réactions:

couleur rouge avec le réactif de Schiff.

précipité jaune avec le réactif de Nessler (E. Pittarelli, Chem. Zentbl., 4,616, 1920) (jaune par l'aldéhyde; blanche par l'acéton).

réduction de la solution de Fehling.

réduction de nitrate d'argent ammonical.

réaction de Jeau.

réaction de Auld et Hantzsch(Ber. Deut. Chem. Ges., 27,514,1888).

réaction de E. Pittarelli(Jour. Pharm. Chem., 23,21,1921).

On ajoute du dimedon dans le distillatum obtenu et obtient des crystaux blancs de l'acétaldomedon fondant à 137—9°. Mélangé avec l'acétaldomedon pur, il fond à la même température.

Présence de l'acide succinique.

Aprés distillation de l'éther de la solution éthére(A), on obtient des crystaux prismatiques fondant à 183° par récristallisation dans l'eau bouillante.

0.1482g. Subst. ont donné 0.2202g. CO_2 et 0.0705g. H_2O.
Calculé pour $C_4H_6O_4$ C 40.67% H 5.08%.
Trouvé C 40.52% H 5.28%.

Présence de l'acide lactique(racémique).

Par le traitement avec le carbonate de calcium, on obtient des crystaux longues aiguilles de lactate de calcium. Pour analyser il a été desséché à 110°.

0.4193g. Subst. ont donné 0.2675g. $CaSO_4$.
Calculé pour $C_6H_{10}O_6Ca$ Ca 18.34%
Trouvé Ca 18.62%

Par le traitement avec le carbonate de zinc, on obtient le lactate de zinc cristallisé en rhombique et on le sèche à 110° pour l'analyse.

0.2552g. Subst. ont donné 0.0845g. ZnO.
Calculé pour $C_6H_{10}O_6Zn$ Zn 26.75%.
Trouvé Zn 26.60%.

Rotation optique en solution aqueuse et eau de cristallisation du lactate de zinc:

dissout 0.4823g. Subst. dans 30g. d'eau. L=1dm: (α) = 0.
0.2296g. Subst. ont perdu à 110° 0.0412g. H_2O.
Calculé pour $(C_3H_5O_3)_2Zn \pm 3H_2O$ H_2O 18.18%……(α)D= 0.
" " " $\pm 2H_2O$ H_2O 12.89%……(α)D= ±9°.
Trouvé H_2O 17.90%.

Présence de l'acide formique et de l'acide acétique.

On récristallise les sels de barium des acides volatils(C) dans l'eau et sèche à 110°.

0 3765g. Subst. ont donné 0.3412g. $BaSO_4$.

Calculé pour	$(HCOO)_2Ba$	Ba 60.42%.
" "	$(CH_3COO)_2Ba$	Ba 53.97%.
" "	$(C_2H_5COO)_2Ba$	Ba 48.55%.
Trouvé		Ba 53.42%.

On acidifie les sels de barium et distille. On trouve la présence de l'acide formique dans ce distillatum avec les réactions suivantes:

réduction de la solution du $HgCl_2$(précipité blanche du HgCl).

réduction de nitrate d'argent ammonical(miror d'argent).

On ajoute l'oxyde mercurique jaune dans ce distillatum et chauffe pour décomposer de l'acide formique, on filtre, on évapore doucement.

On récristallise dans l'eau et sèche dans vide sur de l'acide sulfurique jusju'à poids constant.

0.6226g. Subst. ont donné 0.4500g. HgS

Calculé pour	$(CH_3COO)_2Hg$	Hg 62.96%.
" "	$(C_2H_5COO)_2Hg$	Hg 52.48%.
Trouvé		Hg 62.43%.

EXPERIENCE. II. Relation entre la nourriture azotée et les produits de la fermentation.

Nourriture azotée·········Les quantités differentes des $(NH_4)_2SO_4$, KNO_3, peptone et asparagine, chacun en comprenant 0.1, 0.3, 0.5 et 1%, sont dissoutes dans la solution nutritive.

J'ai déterminé de la solution cultivante fermentée:

1° L'alcool éthylique(fig. 3), par la méthode du distillation.

2° L'acide volatil (fig.5).. On distille 50c.c. de la solution cultivante à la vapeur d'eau jusqu'à 200c.c. du distillatum et titre avec l'alcali titré.

3° L'acide non volatil(fig.4). On titre la solution cultivante fermentée avec l'alcali titré et diminue de cette acidité déterminée l'aciditéde l'acide volatil précédent et l'acidité primitive de la solution nutritive in-fermentée.

4° La glucose(fig.1), par la méthode de Bertrand.

5° Poids du champignon(fig.2).

EXPERIENCE. 111. Sur la zymase.

Par la méthode de macération de A. Lebedeff(Ann. de Inst. Past., 26,7,1912) J'ai obtenu le résultat positif, mais Je mettrai cette publication à l'autre jour.

CONCLUSION.

1° Comme produits de la fermentation avec ce champignon, se produisent en grand partie l'alcool éthylique, puis l'acide succinique, ensuite l'acide lactique et l'acide acétique; en petite quantité l'aldéhyde acétique et l'acide formique.

2° Entre les quantités differentes······0.1—1%······des matière azotée la quantité la plus favorable pour produir l'alcool éthylique est de 1% de la solution nutritive. Et pour produire les acides, J'ai trouvé que 0.1% de matière azotée est la plus favorable.

ON COLOUR REACTIONS OF DIBASIC ACIDS OF FATTY SERIES, ESPECIALLY OF FUMARIC ACID.

By Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

(Received June 28th., 1926.)

On the colour reaction of fumaric acid as far as we know just one[1] is acpuainted with to give a red colouration when the acid is reacted with diazobenzenesulphonic acid. However the delicacy of it is not sufficient enough to be relied upon as a sole reaction. The colour reactions described below offer a more satisfactory means of identification.

FIRST REACTION.

As the reagents required are resorcinol and sulphuric acid, the procedure is already known in the case of succinic acid.[2]

A piece of fumaric acid of the size of a pin's head is placed in a test tube with about ten times its quantity of powdered resorcinol and 1–1.5 c.c. of concentrated sulphuric acid then heated gently in the beginning and later briskly until it attains nearly 190–195°C. Whereby the liquid becomes red with a green fluorescence. After cooling add some water and heat again.

Cool well and a few drops of the reaction products are introduced drop by drop into ammonia, which colours red with a green fluorescence.

This reaction is to be given by the dilute solution of fumaric acid, thus :– A few drops of a 0.1 % solution of fumaric acid placed in a test tube with equal drops of a 0.1 per cent solution of resorcinol and 1–1.5 c.c. of concentrated sulphuric acid gives a liquid which on treating as mentioned above shows a yellow colour with green fluorescence.

Anhydride : i. e. maleinic anhydride gives quite the same colouration as met with by crystals of fumaric acid.

Succinic acid gives a yellow colouration in the first part of the reaction and when introduced into ammonia it changes to a green fluorescent liquid.

Malic acid on treating as above gives a liquid which on heating becomes at first yellow and then crimson-red even after the addition of water. A blueish violet colour is produced by adding 2 or 3 drops of this crimson-red colour fluid into ammonia.

This colouration is quite specific to malic acid and may distinguish this

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(1) Hans Einbeck : Zeit. f. Phys. Chem. Bd. 90, S. 306, 1914.

(2) L. Rosenthaler : Nachweis Org. Verb. S. 331.

from succinic- and fumaric acids.

Tartaric acid. Mohler's reaction(3) for the detection of the acid is analogous to ours in applying the same reagents, although we applied a higher temperature than in his case.(4) No fluorescence is observed when the reaction product is added to ammonia.

Citric acid. Our reaction is a new one to this acid. On treating the crystals of citric acid of the size of a pea as in the case of tartaric acid a liquid is obtained which on heating becomes at first pink-red and then yellow by a prolonged heating. Pink-red colour recovers when it is diluted with water. Finally when a few drops of the liquid is added to ammonia the latter takes a blueish-green fluorescence with violet shade, this is specific to the citric acid.

Beside these dibasic acids gluconic-, mucic-, saccharic-, and lactic acids behave in a very similar way. These reactions will be described in the other paper.

SECOND REACTION.(5)

This is based on the colour produced when the acid to be tested is reacted with α-naphtol in the presence of concentrated sulphuric acid.

The procedure is quite same as it is mentioned in the first reaction, where resorcinol is used.

Fumaric acid. This acid gives a green colour when the reagents are just mixed. By gently heating it becomes at first yellow then pink-red and finally brown-red which changes to an opaque brown liquid after a long standing. Red colour is restored by the addition of water to the liquid. When this liquid, after shaking is introduced drop by drop into ammonia a yellow-green colouration is perceived. After standing the reddish shade intensifies with fluorescence.

The change of reaction given by the other acids will be shown in the table below :-

Acid.	When they are mixed.	By heating.	Colour produced by ammonia.
Succinic acid.	Green.	Dark and light red.	Light greenish-yellow with fluorescence.
Malic acid.	——	Crimson-red and by addition of water it alters to opaque fluid.	Red with fluorescence.
Tartaric acid (Pinĕrũa's reaction :- First blue then green)	Green.	Yellow → light pink red yellow → greenish blue	Light yellow.
Citric acid.	——	Green → crimson-red.	Pink red.

THIRD REACTION.

In this case β-naphtol is used instead of α-naphotl employed in the second reaction. The first part of this reaction is already demonstrated by Pinêrŭa(6) to detect citric acid, which gives a blue color according to his description. For citric acid however the delicacy of the reaction is not sufficient enough as we have experienced with fumaric acid.

A small piece of fumaric acid of the size of pin's head is put in a test tube with β-naphtol and sulphuric acid.

The yellowish-green mixture on heating first colorless then dark-red and finally turns to a dark-green opaque liquid. After cooling and diluting with water, the red color goes into the aqueous layer leaving a green layer underneath it. On shaking the green color is restored with fluorescence which increases when the liquid is added to ammonia.

The colourations given by the other acids by this reagent are tabulated below :-

Acid.	When they are mixed.	On heating.	By adding water.	Colour given by ammonia.
Succinic acid.	Yellowish-light green colouration	Becomes colourless then dark violet and finally greenish violet (Less delicate than fumaric acid).	Water colours yellow.	Green fluorescent fluid.
Malic acid.	Colourless or light greenish yellow.	From light yellowish to red. A fluorescence while yellow. (Distinction from lactate).	Red.	Light yellow with fluorescence.
Tartaric acid.	Colourless.	First green then to dark bluish-green.	Darkness increases.	Coloured yellow with fluorescence.
Citric acid.	——	Light dark blue. Green after cooling. (Blue after Piñerŭa).	Becomes yellowish.	Light green with fluorescence.

FOURTH REACTION.

This reaction is given by hydroquinone and sulphuric acid. The reaction is quite same as in the foregoing reactions. Fumaric acid gives no color

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(3) Mohler's reaction : Bull. Soc. chim. France [3] 4 (1890), 728.

(4) Mohler heated to 130°C, but in our case it should be heated to 190-195°C.

(5) Pinêrŭa's (1897) Colour reaction of tartaric acid which becomes first blue then green when reacted with α-naphtol and sulphuric acid.

just when the reagents are mixed. On heating gently at first a light-red then a dark-red color appears. By the addition of water it changes to yellow. The end color reaction given when the product is added to ammonia is yellow. This reaction is especially preferable to identify succinic acid from fumaric acid which accompanies the former as an impurity.

Following table shows a distinction of colors given by the other acids:-

Acid.	When they are mixed.	On heating.	By adding water.	Colour given by ammonia.
Succinic acid.	Colourless.	First yellow then dark green (Distinction from fumaric acid.)	Dark-yellow.	Fluorescent yellowish-green (Distinction from fumaric acid.)
Malic acid.	Colourless.	First yellow then blood red.	Redness increases.	Dirty yellow.
Tartaric acid.	Colourless.	First yellow with evolution of gas, then reddish-yellow and finally crimson red.	——	Orange yellow.
Citric acid.	Colourless.	Yellow at first then dark green.	——	Yellowish-green with fluorescence.

SUMMARY.

1) The first reaction given by dibasic acid of fatty series in the presence of resorcinol and concentrated sulphuric acid, is a quite new one as to the fumaric acid.

Fumaric acid gives red-color with green fluorescence and by this color is distinguishable from succinic acid which gives just a green fluorescence by the same treatment.

The colourations given by malic, tartaric and citric acids distinguish them from each other most conveniently.

2) The color reaction given by α-naphtol and concentrated sulphuric acid (second reaction) is a new one for fumaric acid (green to red). Succinic acid shows less delicacy than fumaric acid. So it is most conveniently advisable to use this reaction for the identification of either fumaric or succinic acid, especially when the former is present in succinic acid as an impurity. Tartaric acid gives no fluorescence in the last colouration in this reaction as experienced in the first reaction. Citric acid may be distinguished from other acid only by the absence of fluorescence in the last colour.

3) Green colour with fluorescence given by fumaric acid in presence of β-naphtol and sulphuric acid is a new reaction as far as related to this acid. As to succinic, malic, tartaric and citric acids the colouration given by the same procedure is quite specific to each of these acids. The distinction of

succinic acid from fumaric exists in the purplish tinge by the former in the stage of heating in the procedure. Both malic and tartaric acids distinguish from fumaric, succinic and citric acids by the fluorescent light yellow colouration in the end of the reaction. Malic acid characterizes itself from tartaric acid by a yellowish-red or a fluorescent yellow colour in the stage of heating and a red colour by the addition of water in the procedure i. e. the colouration due to the latter is dark bluish-green on heating and dark when diluted with water.

4) The fourth colour reaction given by fumaric acid in presence of hydroquinone and sulphuric acid is a new one with regard to this acid. By the same procedure succinic acid gives a fluorescent yellowish-green colouration which is easily recognisable even accompanied with yellow colour to be produced by fumaric acid if present as an impurity. Malic, tartaric and citric acids show a very delicate and characteristic colouration by this procedure.

(This is reported already in "Journal of the Agric. Chemic. Society of Japan." Vol. No. 14, 1925.1)

ON THE PRESENCE OF THE URIC ACID IN THE PUPA OF TUSSAH SILKWORM (ANTHEREA PERNYI).

By Jiro KATO.

(From the Central Laboratory, S. M. R. Co. Dairen, South Manchuria.)

(Received Aug. 9th., 1926.)

There are many researches on the chemical constitution of silkworm at different stages of its metamorphosis, but no research on tussah silkworm. So we tried to study the chemical constitution of the pupa of tussah silkworm, and learned that the pupa contains a tolerably large quantity of uric acid. We determined its uric acid content by estimating the soluble matter, soluble nitrogen, and uric acid form nitrogen at the different stages of pupa, and the uric acid content was found to be about 1 % at the beginning and 4% at the end.

As tussah silkworm passes a winter in the pupa stage, this stage durates about 200 days and the metabolism is carried on without taking food. Therefore, one can imagine that there is increase of the uric acid content in the pupa body as experienced in this work. It is very interesting as a new utilizing method of the pupa, if the uric acid is prepared in relatively good yield such as 4% or so.

EXPERIMENTAL PART.

1. Sampling.

We bought tussah silk cocoon at Antung, Oct., 1925. At the intervals of 30 days, some pupa were taken out from the cocoons & only those with healthy appearance were weighed, dried powdered & put to the analysis. The date in which the sample was taken is as follows :–

No.	1	2	3	4	5	6	7
No. of days							
Date.	Oct.	Dec.	Nov.	Jan.	Feb.	Mar.	Apr.
	25.	24.	24.	23.	22.	24.	10–20.
	1925.			1926.			
No. of days after pupation. (rough no.)	30.	60.	90.	120.	150	180.	200–200.

(Remarks :- 1–6. Pupa powder. 7. Moth powder.

2. Total nitrogen, soluble and insoluble nitrogen.

The total nitrogen was determined by Kjeldahl's method. To estimate the soluble nitrogen, 2 gms. of the sample were weighed with hot water, repeatedly and filled up to 2 liters, and a definite quantity of this extract was taken and its nitrogen was determined. The difference of these two nitrogens is calculated as to be the insoluble nitrogen. This experiment gave the following results :–

	1	2	3	4	5	6	7
% in fresh matter.							
Total N.	2.24	2.45	2.52	2.48	2.52	2.62	3.32
Soluble N.	0.80	0.74	0.91	1.02	1.08	1.11	0.58
% in dry matter							
Insol. N.	1.64	1.72	1.61	1.46	1.44	1.51	2.74
Total. N.	10.07	10.75	11.37	11.05	11.34	12.21	11.005
Sol. N.	3.31	3.23	4.12	4.56	4.83	5.18	1.93
Insol. N.	6.76	7.52	7.25	6.49	6.51	7.03	9.12
% in total nitrogen.							
Sol. N.	33.03	30.05	36.24	41.27	42.59	42.42	17.499
Insol. N.	66.97	69.95	63.76	58.73	57.41	57.41	82.53

From the above results, it is distinct that the soluble nitrogen increases according as the course of the pupa stage. The increase of soluble nitrogen may be due to the increase of the substances of lower molecular weight such as the amino acids, bases, soluble proteins, uric acid, & produced by the decomposition of the insoluble nitrogenous matter. But this increase is chiefly due to the uric acid, as it can be proved by other experiment that the amino acids, bases and soluble proteins are not increased markedly.

3. Soluble matter.

Some grms. of the sample were extracted with 500 times of its weight of hot water, and the definite volume of this extract was dried up and weighed. The soluble matter also increases according as the course of the pupa stage. This increase means the formation of the substances of lower molecular weight such as amino acids, bases, proteins and uric acid. Although the increase of the uric acid was not determined in this experiment, one can assume that there was increase of the uric acid.

	1	2	3	4	5	6	7
% in fresh matter.	3.73	4.28	6.38	6.39	6.75	7.38	5.89
% in dry matter.	15.40	18.72	28.82	28.41	30.32	31.37	19.60

4. Uric acid nitrogen.

2 gms. sample was extracted with hot water repeatedly and the extract was filled up to 1 liter. From this extract 400 c.c. were taken and the uric acid nitrogen was determined by Krüger and Schmidts' method (Z. physiol. chem. 75. 1. 1905.). From this experiment we found the increase of uric acid nitrogen and uric acid as shown in the next table.

	1	2	3	4	5	6	7
Uric acid nitrogen.							
% in fresh matter.	0.10	0.11	0.30	0.31	0.34	0.37	0.02
% in dry matter.	0.42	0.48	1.37	1.37	1.57	1.72	0.08
Uric acid.							
% in fresh matter.	0.30	0.33	0.90	0.93	1.02	1.17	0.06
% in dry matter.	1.26	1.44	4.11	4.11	4.71	5.26	0.24

From these experiments it is clearly found that the increase of the uric acid at the pupa stage is within the limit of about 1–4 %, and the increase is due to the decomposition of the higher insoluble introgenous matters, and the preparation of the uric acid from the pupa is possible.

(31th. July, 1926)

ANTINEURITIC PROPERTIES OF ADSUKI BEAN.

By Eiji Takahashi and Kiyoshi Shirahama.

(Received July 13th., 1926.)

1. Adsuki bean is held in Japan to have a curative effect on beri–beri.

Therefore, the content of the antineuritic vitamine in the bean was studied with its alcohol and water extract upon pigeon, with no marked efficacy. But the normal growth was observed by feeding them with the whole grain of the bean, showing apparently the sufficient content of vitamine B. It is understood that these results must be confirmed with the experimental animals other than pigeon.

2. Allantoin was isolated from the bean and studied of its action uppon the polyneuritic pigeon, but no marked effect was observed.

3. The curative potency of the bean on beri-beri will propably be due to another ingredients than vitamine in it. Saponin, found in the bean, may be reckoned as one of them as well.

ÜBER DIE STICKSTOFFHALTIGEN EXTRAKTIVSTOFFE DER PFERDEHODEN.

Von Kiyohisa YOSHIMURA und Yoshiharu HIWATARI.

(Eingegangen am 2. Aug., 1926.)

Studien über die Bestandteile der Stierhoden sind schon seit langem von vielen Autoren unternommen. L. Leibfreid * hat aus den Stierhoden Dimethylguanidin isoliert.

Bei unseren eingehenden Untersuchungen konnten wir in den Pferdehoden Xanthin, Leucin und β–Alanin in kleiner Menge, Cholin und Methylguanidin reichlich isolieren.

1.9 kg. zerhackte Masse der kurz nach der Kastration entnommenen, von der Kapsel befreiten Füllenhoden wurden mit heissem Wasser wiederholt extrahiert, die Auszüge mit Bleiessig gereinigt, dann mit Schwefelwasserstoff entbleit.

Das Filtrat vom Bleisulfid wurde im Vakuum stark eingeengt, wobei schied sich 0.3 g. Xanthin aus.

(I) Der Phosphorwolframsäureniederschlag. Die Mutterlauge vom Xanthin wurde mit Schwefelsäure angesäuert und mit Phosphorwolframsäure gefällt.

(a) Die aus dem Phosphorwolframat in gewöhnlicher Weise dargestellte alkalische Flüssigkeit, welche freie Basen enthielt, wurde mit Salpetersäure

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* L. Leibfreid; Zeitschr. f. Physiol. Chem. 139, 82, 1924.

neutralisiert und dann mit Silbernitrat versetzt, wobei ein gelbbrauner Niederschlag entstand; die Menge desselben war aber so gering, dass er zur weiteren Untersuchung nicht ausgereicht hätte.

(b) Der Silbernitrat-und Barythydratniederschlag.

Das Filtrat vom Silbernitratniederschlag wurde mit Silbernitrat und Barythydrat in mässigem Überschusse versetzt.

Der gebildete dunkelbraune Niederschlag wurde mit warmer Salzsäure zersetzt; das Filtrat vom Silberchlorid wurde mit Phosphorwolframsäure gefällt.

Die so gewonnene stark alkalische Flüssigkeit wurde mit überschüssiger Salzsäure angesäuert, eingeengt und dann im Vakuumexsikkator stehen gelassen, wobei sich farblose Krystalle ausschieden, die sich nach völligem Austrocknen durch Behandeln mit absolutem Alkohol in folgende Zwei Fraktionen trennen liessen:

1. Die in absolutem Alkohol unlösliche Fraktion.

Das chlorid wurde in das Chloraurat übergeführt; das Chloraurat bestand aus blasgelben Nädelchen mit dem Schmelzpunkt 150–158°C (β-Alaninchloraurat).

2. Die in absolutem Alkohol lösliche Fraktion.

Das aus einem Teil des Chlorides dargestellte Chloraurat bildete gelbe Prismen mit dem Schmelzpunkt 200–204°C (Methylguanidinchloraurat).

Das aus einem Teil des Chlorides dargestellte Pikrat bildete gelbe Nädelchen, die bei 195°C schmolzen (Methylguanidinpikrat).

(c) Das Filtrat vom Silbernitrat-und Barythydratniederschlag.

Das Filtrat vom Silbernitrat-und Barythydratniederschlag wurde in gewöhnlicher Weise wieder mit Phosphorwolframsäure gefällt.

Die aus diesem Niederschlag erhaltene alkalische Flüssigkeit wurde mit überschüssiger Salzsäure angesäuert, eingeengt und dann im Vakuumexsikkator krystallisieren gelassen.

Die so erhaltene hygroskopische Krystallmasse wurde in mit alkoholischer Sublimatlösung gefällt. Die Quecksilberdoppelsalze wurden mit Schwefelwasserstoff zersetzt, die gewonnene Chloride im Vakuumexsikkator stehen gelassen, wobei sich farblose hygroskopische Nadeln ausschieden.

Ein Teil des Chlorides wurde in das Chloraurat übergeführt.

Das Chloraurat bestand aus orangegelben Blättchen mit dem Schmelzpunkt 260–254°C (Cholinchloraurat).

(II) Das Filtrat vom Phosphorwolframsäurefällung (I) wurde mittels Barythydrat von Schwefelsäure und Phophorwolframsäure befreit, der Überchuss des Baryts mittels Schwefelsäure beseitigt und im Vakuum stark

eingedampft, wobei sich kleine Menge von Krystalle ausschieden (Leucin).

Also aus 1.9 kg. frischen Hoden wurden isoliert.

Xanthin	0.3 g.
Xanthin–Alanin (Chloraurat)	0.3 g.
Methylguanidin (Chlorid)	o.2 g.
Cholin (Chloraurat)	1.5 g.
Leucin	Wenig.
NH_4Cl	0.8 g.

ON THE PHYSICAL PROPERTIES OF "ASAHI PROMOLOID"

By Hideo KANEKO.

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(Received July 30th., 1929.)

INTRODUCTION.

The soil, upon which plants take root and thrive, is considered to be a colloid as well as the plants themselves, hence there exist a great common stream of energy between these two worlds.

Recently E. Bottini (Ann. di chim. Appli. **16**, 29 (1926)) made public a very instructive treatise with respect to permeability, capillarity and absorbing power of soils in the presence of Promoloid at the Torino Agricultural Experimental Station in Italy.

He observes that the permeability and capillarity of soils are diminished appropriately in the presence of Promoloid and its absorbing power for ammonium chloride is increased.

I made also a series of experiments on the same subject during the summer in 1924. As the results obtained by me agree with those of Bottini in main respects, I will discuss shortly my results in the following.

Promoloid has good effects upon the growth of many plants in Japan. Especially sugars, starch and phosphoric acid contents in plants are fairly increased by it.

Y. Matsuyama and co-workers (Report of Research Laboratory, Asahi

Glass Co., Ltd., 11, 13, 15, in 1925) showed that Promoloid has a substituting action for phosphoric acid. It is known that colloidal silica, one of the components of Promoloid, displays many important photochemical and biochemical actions; the inversion of cane sugar (Albert et A. Mary, Compt. rend. **167**, 644–1918; R. T. Dufford S. Calvert and D. Nightingale, J. Am. Chem. Soc. **45**, 2058–1923), the substitute action for P_2O_5 in plants (D. R. Nanji and W. S. Shaw, J. Soc. Chem. India, **44**, 1–1925), Selective positive adsorption of PO_4 ion (R. C Wiley and N. E. Gordon, Soil Scie. **14**, 441–1922), positive action for photochemical decomposition of silver bromide (R. Schwarz und H. Stock, Zeit. Anorg. and Allg. Chem., **129**, 41–1923) etc.

The other component magnesium or magnesium oxide also displays many special actions. The former is contained in chlorophyll as an essential constituent which plays a great rôle in the assimilation and seemes tosynthesise vitamine A from inorganic compounds. (K. H. Coword and J. C. Drummond, Biochem. J. **15**, 530–1921.)

In practice, green color of leaves of plant is deepened by Promoloid and 80 % aceton extract of such leaves maintains its green color for a long time.

Magnesium combines with nitrogen at high temperature, but I consider that, under a special catalytic action of ferments or colloidal substances, it may have some favorable relations with nitrogen containing compounds such as protoplasmic protein even at room temperature.

The magnesium oxide has a favorable action for the absorption of phosphoric acid under colloidal state, disperses the soil colloids in smaller particles and makes nitrification more vigorous in the acidic soil.

As physical properties of soil I took into the consideration ;–the dispersion of soil particles, absorbing capacity of water, absorbing power of fertilizer and permeability of water. The effect of Promoloid was observed for these factors.

EXPERIMENTAL PART.

[*I*] *Field soils*:

300 grams of field soil in natural state as far as possible were put in a large glass funnel which was stopped at its bottom by absorbent cotton. It was moistened properly with rain water and left standing for a day. On the next day, a definite velume (100 c.c.) of rain water or solution containing fertilizer were poured upon the soil. The volume of water passing through it was measured in a cylinder at definite intervals.

(a) Dry sandy field soil near the Tamagawa, Tokyofu.

Dry state, very coarse grain;

		V_1(c.c.)	V_2(c.c.)	V_3(c.c.)
(I)	Rain water (100 c.c.)	19	37.5	38
(II)	Water + Promoloid (0.5 c.c.)	17	33.0	37.5
(III)	Water + 2 g. $(NH_4)_2SO_4$	19	35.5	36
(IV)	Water + 2 g. $(NH_4)_2SO_4$ + Promoloid	12	28.0	38
(V)	Water + 2 g. KNO_3 + Promoloid	14.5	31.5	31.5
(VI)	Water + 2 g. Na_2HPO_4 + Promoloid	15	34.0	34.5

V_1 .... Volume of water passing through soil after 10 minutes.
V_2 .... 〃 〃 〃 20 minutes.
V_3 .... 〃 〃 〃 30 minutes.

(b) Field soil at Mito:

Wet state, very coarse grain;

		V c.c. (after 5 minutes)
(I)	Rain water	92
(II)	Water + Promoloid (0.5 c.c.)	90
(III)	Water + 2 gr. $(NH_4)_2SO_4$	92
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	99
(V)	Water + 2 gr. KNO_3 + Promoloid	97
(VI)	Water + 3 gr. Na_2HPO_4+ Promoloid	13

(c) Field soil at Yoyogi, Tokyofu:

dry state, coarse grain;

		V c.c. (after 30 min.)
(I)	Rain water	80
(II)	Water + Promoloid (2 c.c. ten times diluted solution)	73
(III)	Water + Oil cake (2 gr.)	61
(IV)	Water + 2 gr. Oil cake + Promoloid	71.0
(V)	Water + 2 gr. $(NH_4)_2SO_4$	66.0
(VI)	Water + 2 gr. $(NH_4)_2SO_2$ Promoloid	74.5
(VII)	Water + 2 gr. Calcium superphosphate	64.0
(VIII)	Water + 2 gr. Ca-superphosphate + Promoloid	73.0

(d) Field soil, at Kumagai, Saitama:

Wet state, small grain, compact condition;

		V c.c. (after an hour)	V c.c. (after 2 hours)
(I)	Rain water	9.0	17.0
(II)	Water + Promoloid	8.2	16.0
(III)	Water + 2 gr. $(NH_4)_2SO_4$	23	44
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid (0.5 c.c.)	25	48.5
(V)	Water + 2 gr. Na_2HPO_4 + Promoloid	8.5	16.5

(e) Field soil at Toyonaka, Osakafu:

Wet state, small grain, compact condition;

		V c.c. (after 1 hour)	V c.c. (after 2 hours).
(I)	Rain water	22	34.0
(II)	Water + Promoloid (0.5 c.c.)	20.1	32.0
(III)	Water + 2 gr. $(NH_4)_2SO_4$	26	37.5
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	41	42.9
(V)	Water + 2 gr. Na_2HPO_4 + Promoloid	20	32.0

(f) Sandy soil at Tamagawa, Tokyofu:

Wet state, coarse grain, compact condition:

		V_1	V_2	V_3
(I)	Rain water	30.5	58.6	84.5
(II)	Water + Promoloid (0.5 c.c.)	31.5	59.5	85.0
(III)	Water + 2 gr. $(NH_4)_2SO_4$	33	64	93
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	17	32	46
(V)	Water + 2 gr. KNO_3 + Promoloid.	30	53.5	73.5
(VI)	Water + 2 gr. Na_2HPO_4 + Promoloid	26	47.5	66.5

V_1……………………after 10 minutes.
V_2……………………after 20 〃
V_3……………………after 30 〃

[*II*] *Muddy field soils or Rice field Soils*:

300 grs. of soil, salts and 100 c.c. water were mixed well in a beaker, frequently stirred to a muddy form and left standing for 3 days. The mixture was then poured into a large glass funnel described above and its permeability of water was measured as before.

(a) Rice field soil, Yoyogi; Tokyofu:

Muddy state, small grain;

		V c.c. (after a day)
(I)	Rain water	93.5
(II)	Water + Promoloid (2 c c. ten times diluted solution)	96.0
(III)	Water + Oil cake (2 gr.)	98
(IV)	Water + 2 gr. Oil cake + Promoloid	94.5
(V)	Water + 2 gr. $(NH_4)_2SO_4$	97.5
(VI)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	95
(VII)	Water + 2 gr. Calcium Superphospate	98
(VIII)	Water + 2 gr. Ca-Superphosphate + Promoloid.	95

(b) Muddy rice field soil, Kumagai, Saitama:

Muddy state, small grain:

		V c.c. (after a day)
(I)	Rain water	95
(II)	Water + Promoloid (0.5 c.c.)	96
(III)	Water + 2 gr. $(NH_4)_2SO_4$	96.5
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	83.5
(V)	Water + 2 gr. Na_2HPO_4 + Promoloid	92.5

(c) Muddy rice field soil, Nakasu, Nagano:

Muddy form, very small grain, compact condition.

		V c.c. (after 1 day)	V c.c. (after 3 days)
(I)	Rain water	5.0	30
(II)	Water + Promoloid (0.2 c.c.)	5.3	32
(III)	Water + 2 gr. $(NH_4)_2SO_4$	5.5	33
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	3.5	37

(d) Muddy field soil, Hayama, Kanagawa:

Muddy form, very small grain, compact condition.

		V c.c. (after a day)
(I)	Rain water	9.0
(II)	Water Promoloid (0.5 c.c.)	9.5
(III)	Water + 2 gr. $(NH_4)_2SO_4$	23.0
(IV)	Water + 2 gr. $(NH_4)_2SO_4$ + Promoloid	15.5

From the above results we obtain the following table.

V_o ………… Volume of water passing through the soil after t minutes.
V_o' ………… Volume of water containing Promoloid after t minutes.
V ………… Volume of water containing ammonium sulfate after the time t.
V' ………… Volume of water containing ammonium sulfate and Promoloid after the time t.

		V_o	V_o'	$\frac{V_o'}{V_o}$	V	V'	$\frac{V'}{V}$
(I)	(a)	19	17	0.90	19	12	0.63
		37.5	33	0.88	35.5	28	0.79
		38	37.5	0.98	36	38	1.11
	(b)	92	90	0.62	92	99	1.07
	(c)	80	73	0.91	66	71.5	1.12
	(d)	9	8.2	0.91	23	25	1.09
	(e)	22	20.1	0.91	36	41	1.13
		34	32	0.94	37.5	42.9	1.12
	(f)	30.5	31.5	1.03*	33	17	0.51*
		58.5	59.5	1.01*	64	32	0.50*
		84.5	85.0	1.00*	93	46	0.50*
(II)	(a)	93.5	96	1.02*	97.5	95	0.97*
	(b)	95	96	1.01×	96.5	83.5	0.85*
	(c)	5	5.3	1.06*	5.5	3.5	0.63*
		30	32	1.06*	33	37	1.12
	(d)	9	9.5	1.05*	23	15.5	0.67*

In all field soils except wet sandy soil (f) the permeability of water through the soil diminishes in the Presence of promoloid. This agrees with Bottini's result. But, the permeability of water which contains ammonium sulfate increases on the contrary in the presence of Promoloid; the increment of permeability being about 10 percent. But oppositely, in wet sandy field

Fig. 1

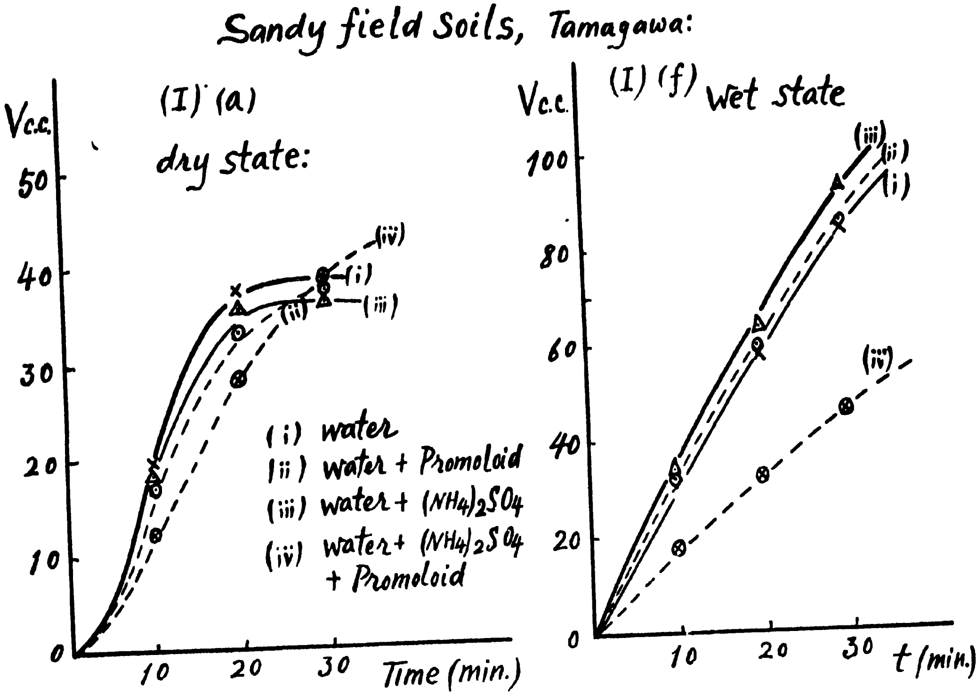


Fig 2

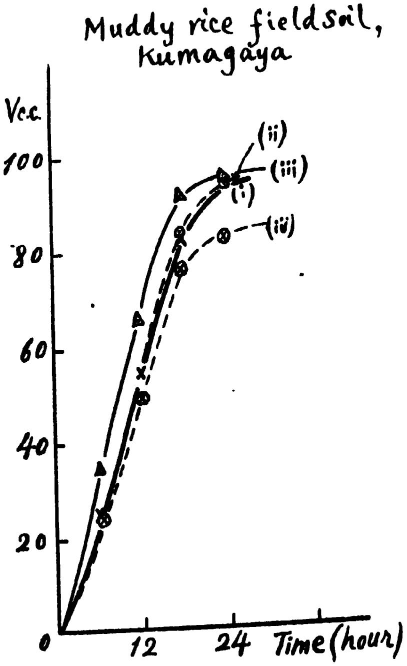
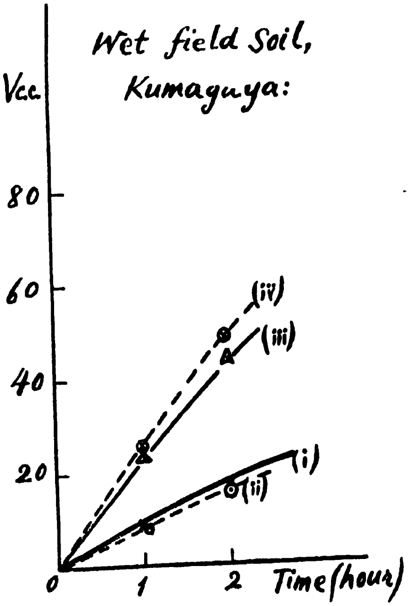


Fig. 3



soils and muddy rice field soils the permeability of water increases after a long time in the presence of promoloid and the permeability of water which contains ammonium salts diminishes greatly.

In all cases it was found that the quantity of ammonium in the filtrate is far less in the presence of Promoloid. Hence we know that the Promoloid has a strong absorbing power for ammonium sulfate and prevents it to flow out with water. And it diminishes the Permeability of water in coarse field soils and increases it in muddy rice field soils which fill with water during about four months.

This makes active for the renewal the circulation of water and to absorb easily rain water containing small quantity of nitrogen compounds and radioactive substances. The surface of soil where promoloid was given becomes very rough and seemes to help the developement of roots by facititating the ventilation of the air through it.

SUMMARY :

(1) Generally speaking the permeability of water through the soil diminishes fairly in the presence of Promoloid in the field soils and increases in the muddy rice field soils.

(2) On the contrary the permeability of water containing ammonium sulfate increases in the presence of Promoloid in the former case and diminishes in the latter case.

This is because it easily absorbs rain, fertilizers and will keep out probably against the decomposition of salt near the surface and its outflow.

(3) The Promoloid easily absorbs ammonium and potassium salts from the solution and reserves them in the soil. When the contents of ammonium and potassium ions in the filtrates were compared, they were by far the less in those come from the soils given with Promoloid. Hence it assists the absorption of manures through roots and give the favorable effects for the growth of plants.

(4) The surface of soils to which the Promoloid was administered, becomes generally very coarse, so it will help the entrance of air and rain into the soil.

The author is indebted to Prof. Dr. Y. Shibata, Dr. M. Fukui, and Dr. M. Namba in the research laboratory, Asahi Glass Company, for their kind advices and to Mr. U. Sakurai, Professor of Physics in the Keio Medical College, for his kind assistance.

ON THE NUTRITIVE VALUE OF SYNTHETIC FATS CONTAINING OXY-FATTY ACIDS.

By Junichi OZAKI.

Biochemical Laboratory, Faculty of Agriculture, Tokyo Imperial University.

(Received June 25th., 1926.)

In continuation to the previous report(1) the author carried out the feeding experiment with synthetic fats containing oxy-fatty acids to compare their nutritive value with other kinds of fats. Oxy fatty acids, except ricinoleic acid, occur in natural fats only in negligible quantity, and so they seem to play only a subordinate rôle in the animal nutrition. But, according to Knoop, β-oxy acids are formed as the intermediate products of the successive degradation of long fatty acid chains. Further, the fate of other oxy acids having one or more OH group at different position has never been studied thoroughly. The feeding experiments with these fats will therefore contribute something to the knowledge of the fat metabolism in the animal body.

The author has prepared 13 kinds of oxy acids, which were carefully purified and their melting-and boiling-points as well as molecular weights thoroughly examined.

The preparation of triglycerides from these oxy acids was carried out by means of Twitchell's reagent as described in the first report.(1) But, lactin, dioxyundecylin, dioxystearin and trioxystearin could not be prepared by this method, probably these oxy acids being dehydrated by Twitchell's reagent and subsequently decomposed on heating ; and so they were previously acetylated and converted into acetylated glycerides.

Beside these synthetic fats, the author used also the mixture of these oxy acids and glycerine. Furthermore, the nutritive value of ricinolein, acetyl ricinolein and ricinoelaidin were compared one another. The method of feeding used here was exactly the same with that of the former experiment,(1) i. e. the young rats were first fed with a limitted quantity of basal diet (9 grams per day per rat), and when the growth was stopped they were supplied with the test diet containing 5, 10 and 20% of the fats under examination to the basal diet, respectively, and the growth induced thereby were compared each other. These experiments were carried out in the same season and possibly under the same condition.

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(1) This Journal. Vol. II. No. 1, 1926.

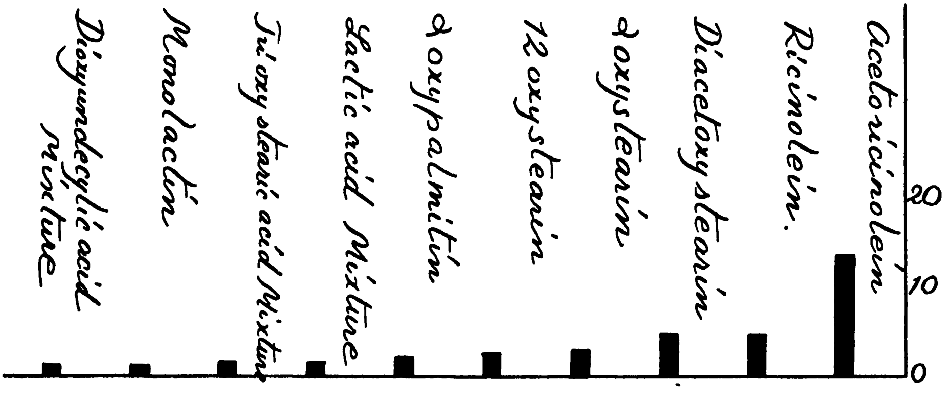
The results thus obtained were as follows:

(1) The growths induced by adding 5% of each fat to the basal diet were shown in the following order:

1.	Acetylricinolein	13.5g.	2.	Ricinolein	4.5g.
3.	Diacetoxystearin	4.5g.	4.	α-Oxystearin	3g.
5.	1 2-Oxystearin	2.5g.	6.	α-Oxypalmitin	2g.
7.	Lactic acid & Glycerin	1.5g.	8.	Trioxystearic acid & Glycerin	1.5g.
9.	Monolactin	1g.	10.	Dioxyundecylic acid & Glycerin	1.0g.
11.	Triacetoxystearin*		12.	Dioxystearic acid and Glycerin*	
13.	Diacetoxyundecylin*		14.	α-Oxyheptylin*	
15.	α-Oxymyristin*		16.	Ricinoelaidin*	

FIG. 1.

Showing the increase of body weight after adding 5 % of Sample to the basal diet,



(2) By adding 10% of each fat:

1.	Acetylricinolein	58.5g.	2.	1 2-Oxystearin	24.5g.
3.	Dioxystearic acid & Glycerin	19.0g.	4.	Monolactin	19.0g.
5.	Diacetoxystearin	18.5g.	6.	α-Oxystearin	17.0g.
7.	α-Oxystearic acid & Glycerin	7.0g.	8.	α-Oxypalmitin	4.0g.
9.	Ricinolein	3.0g.	10.	Dioxyundecylic acid & Glycerin*	
11.	Trioxystearic acid & Glycerin*		12.	Ricinoelaidin*	
13.	Triacetoxystearin*		14.	Diacetoxyundecylin*	
15.	Lactic acid & Glycerin*		16.	α-Oxyheptylin*	
17.	α-Oxymyristin*				

(3) By adding 20% of each fat:

1.	Acetyl ricinolein	63.0g.	2.	Dioxystearic acid & Glycerin	18.0g.
3.	1 2-Oxystearin	16.5g.	4.	Diacetoxystearin	4.5g.
5.	α-Oxystearin	2.5g.			

FIG. 2.

Showing the increase of body weight after adding 10% of Sample to the basal diet.

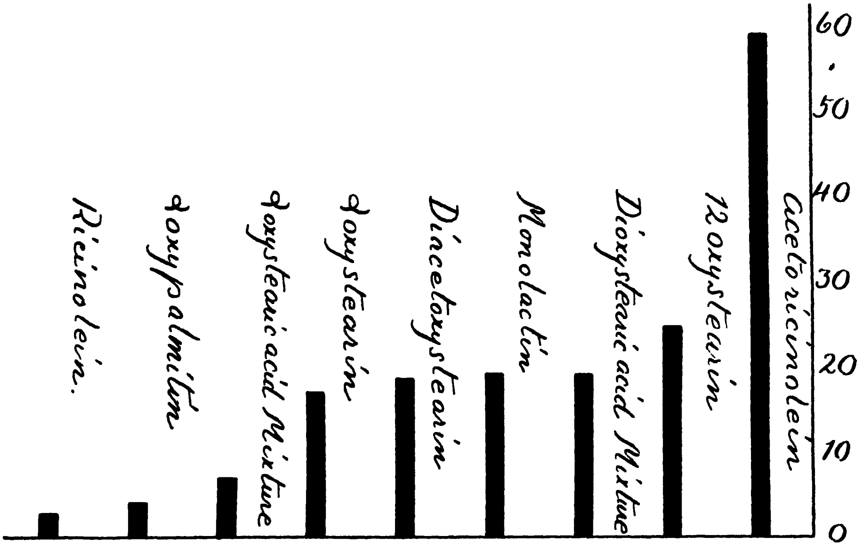
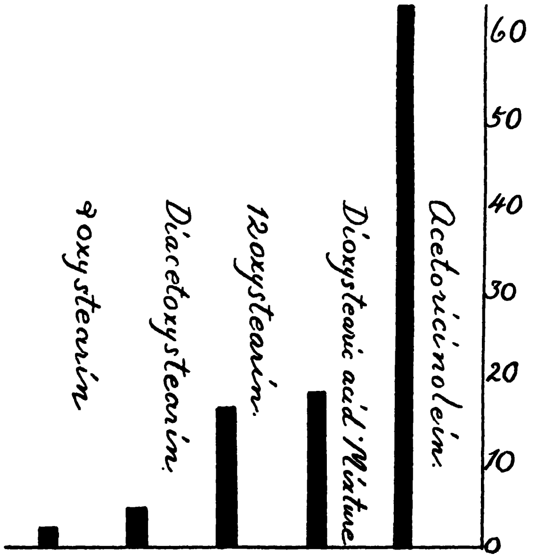


FIG. 3.

Showing the increase of body weight after adding 20% of Sample to the basal diet.



In the above tables, those fats assigned with asteriks* were found to have no nutritive value, and some of them being decidedly noxious of special interest is the fact that acetyl ricinolein was far better than ricinolein and moreover that 1 2-oxystearin prepared by the hydrogenation of ricinolein gave a higher nutritive value than ricinolein itself.

This might be due to the breaking of the fatty acid chains at different points, yielding thereby different products, which would naturally behave differently in the animal body.

The breaking takes place probably in the following way:-

1) $\mathrm{C_6H_{13}CH\ (OH)\ CH_2CH:CH\cdot(CH_2)_7COOH} \longrightarrow$

Ricinolein $\mathrm{C_6H_{13}CHO + CH_3CH:CH\cdot(CH_2)_7COOH}$

Undecyleic acid.

2) $\mathrm{C_9H_{13}CH\ (O\cdot CO\ CH_3)\ CH_2CH:CH\cdot(CH_2)_7COOH} \longrightarrow$

Acetylricinolein $\mathrm{C_6H_{13}CH\ (O\cdot CO\cdot CH_3)\ CH_2CH-}$

$\mathrm{+\ -CH\ (CH_2)_7COOH}$

3) $\mathrm{C_6H_{13}CH\ (OH)\cdot CH_2CH_2(CH_2)_7COOH} \longrightarrow$

1 2-Oxystearin $\mathrm{C_6H_{13}CHO + CH_3CH_2CH\ (CH_2)_7COOH}$

Undecylic acid.

Among the above mentioned products Δ9 undecyleic acid formed from ricinoleic acid is decidedly noxious as proved by the author in his former experiment,(1) while others from acetylricinolein and 1·2-oxystearin have no noxious effect at all.

It seems that the breaking of the molecules occurs at first, at the point where OH group is attached or where double linking is present.

SUMMARY OF THE RESULTS.

1) The nutritive value of the fats containing oxy-fatty acids differs according to the position of OH group. Thus, for instance, α- and 1 2-oxystearin were found to have quite different values.

2) The fats containing α-oxy acids are inferior to those of the corresponding saturated fatty acids, so it is improbable that α-oxydation occurs in the animal body.

3) The noxious effect of certain α-oxy acids decreases with the increase of the molecular weights. Thus for instance, α-oxyheptylin and α-oxymyristin have stronger toxicity than α-oxypalmitin or α-oxystearin.

4) The nutritive value of oxy-acids depends on the position of OH

group, rather than the number of OH groups in the molecule.

5) The nutritive value of ricinolein is greatly improved by acetylation, but as to dioxyundecylin, dioxystearin and trioxystearin, the acetylation had little effect upon the nutritive value.

6) The improvement of the nutritive value of ricinolein by acetylation may be due to the formation of different products by breaking of its molecule.

7) Ricinolein was found to be better than its solid isomer, ricinoelaidin.

ON THE DISTRIBUTION OF A NEW THIOAMINO-ACID.

by Satoru OHDAKE.

(Abstract from the Original Paper)

(Received Sept 4th., 1926.)

In 1924, U. Suzuki, T. Mori and the author isolated a new sulphur compound from the alcoholic extract of yeast, and gave the empirical formula $C_{11}H_{15}NSO_3$ to it. Boiled with diluted acids, it was hydrolysed easily to Adenin ($C_5H_5N_5$) and a new thiosugar ($C_6H_{12}SO_4$): so the authors concluded, this compound should be adenyl-thiomethyl-pentose. (U. Suzuki, S. Ohdake, and T. Mori :- The Journ. of the Agricultural Chemical Society of Japan. Vol. I No. 2 p. 127-136, 1924 and Biochemische Zeitschrift, B. 154, Heft. 3/6 S. 278-289, 1924.)

On studying further the alcoholic extract of yeast, the author isolated a new thioamino-acid in the following way :- The alcoholic extract of yeast was evaporated under diminished pressure to a syrupy consistence and dissolved in a little water. A concentrated tannin solution was then added, the precipitate thus formed, was collected, decomposed with baryta water and filtered. The filtrate, freed from an excess of baryta, was evaporated to a small volume, when the crystals of adenyl-thiomethyl-pentose separated out which were filtered off. To this filtrate, strong alcohol was added enough to make the alcoholic content of the mixture 80% by volume. The voluminous precipitate thus formed, was filtered by suction, and recrystallised several times from diluted alcohol. The crystals were found to be the mixture of leucin and a sulphur compound, but it was impossible to separate them by fractional crystallisation. It was therefore dissolved again in water, and the saturated solution of mercuric chloride was added. The sulphur compound alone, forming an insoluble salt with it, was precipitated and then decomposed with hydrogen sulphide. This treatment was repeated again and the resulted crystals were recrystallised from diluted alcohol. The yield of the purified compound was 0.6 g. from 334,280 kg. of fresh yeast.

The sulphur compound thus obtained having the emperical formula $C_5H_{11}SNO_2$ is apparently a thioamino-acid as the analytical results of the free compound as well as of its derivatives show.

The purified compound is colourless and crystallises in thin monoclinic plates. Heated in a capillary, it melts at 271-272°C (uncorr.) with decomposition. It is easily soluble in water and in diluted alcohol, but insoluble in ether, benzene etc. Its specific rotatory power is $[\alpha]_D^{16} = -11.77°$ in water. The aqueous solution of this compound gives a violet colour reaction with ninhydrin when warmed, but Millon's, Folin's, and biuret-reaction are all absent. With mercuric chloride, mercuric nitrate, and mercuric sulphate, it gives a white precipitate, but it is precipitated neither by phosphotungstic acid nor by picric acid. Even a boiling strong alkali does not split sulphur from this. compound in a form, detectable by sodium nitroprusside or by lead acetate, while both these reactions are positive, when it is fused with metallic sodium. In contrary to ethyl-cystein this sulphur compound is quite stable against a boiling strong alkali; giving neither ammonia nor ethylmercaptane. (Compare; Brenzinger :- Zeitschrift f. physiol. Chem. XVI. 563, 1892. Neuberg und Mayer :- Zeitschrift. f. physiol. Chem. **44**, 489, 1905.)

The copper-salt Cu $(C_5H_{10}SNO_2)_2$ forms light blue thin monoclinic plates which are somewhat soluble in boiling water but almost insoluble in the cold. Its derivative of *α*-naphtyl-isocyanate $C_{16}H_{18}N_2SO_3$ crystallises in white long needles, melting at 187°C (uncorr). It is almost insoluble in water, ether etc., but dissolves easily in alcohol. Its *β*-naphtalene-sulpho-derivative $C_{15}H_{17}S_2NO_4$ forms white needles, melting at 204°C (uncorr).

From these properties, the compound should be a new thioamino-acid having the formula $(C_3H_7S)-CHNH_2COOH$, besides the known sulphur compounds as cystin, cystein, and taurin etc. (S. Ohdake :- the Journ. of the Agricultural Chemical Soc. of Japan. Vol. I. No. 8. 1925, and Biochemische Zeitschrift. Bd. 161 Heft 4/6, 1925.)

Recently J. H. Muller isolated a new sulphur compound $C_5H_{11}SNO_2$ from hydrolytic products of casein and egg-albumin. (J. H. Muller :- Journ. of Bact. VII p. 309-325. 1922 and Journ. of biol. chem. LVI. No. 1. 1923).

The thioamino-acid, isolated by the author from the yeast-extract, has entirely the same properties with it. For the identification of these two substances, the author prepared the sulphur compound from casein according to Muller's mercuric method :-

Casein was hydrolysed with sulphuric acid, and neutralised with sodium hydroxide solution. To the filtrate, the sulphuric acid solution of mercuric sulphate was added, and the mixture was again neutralised with caustic soda. The precipitate was then extracted with hot 2 % baryta water and filtered by suction. The filtrate freed from mercury and barium was evaporated to a small volume. Then the boiling saturated solution of mercuric chloride was added, using about 30 g. of the reagent for each pound of protein. Standing about 30 hrs., the precipitate was collected and decomposed with hydrogen sulphide. The filtrate freed from mercury was evaporated to dryness in vacuo, and the residual substance was dissolved in water and treated with freshly prepared silver oxide. The silver chloride is filtered off and the excess of silver was removed with hydrogen sulphide. The filtrate from silver sulphide was evaporated to a small volume in vacuo. Finally, three or four volumes of hot alcohol were added to it, and on cooling, the sulphur compound separated as shining crystals. For the purpose of the purification, these crystals were dissolved in about ten times of hot water and treated with mercuric chloride again as described above. The yield of the pure compound was 1.12-1.74 g. from each pound of casein.

As expected, the sulphur compound thus prepared from casein had entirely the same properties with that isolated from yeast-extract, except that its specific rotatory power was a little lower. It was proved that, the compound isolated by Muller was partially raceminized in the course of the extraction with hot baryta water.

The author, further, isolated the same thioamino-acid from the hydrolytic product of yeast-protein by the same treatment. It is clear, therefore, that the thioamino-acid isolated from yeast-extract must have been produced by the autolysis of yeast itself.

Although the existence of non-cystine-protein-sulphur has already been suspected often, it was still an open question if there exist one or more sulphur compounds in protein molecule other than cystin. The isolation of new sulphur compounds from protein should be a sole key to solve this problem.

To know the distribution of the "thioamino-acid," the author worked on the several kinds of protein by the same mercuric method as described above, and isolated the same thio-compound in a pure state though not quantitative.

The yields of the "thioamino-acid" are as follows:-

Casin	0.25–0.39 %
Egg-albumin "Merck"	0.42 %
Blood-fibrin	0.26 %
Beer-yeast	0.008%
Protein from rice-bran	0.002%
Protein from soya-fean	——

From these results, the author has come to the general conclusion that the "thioamino-acid" is very widely distributed and is comparatively abundant in amimal proteins but scanty or even absent in vegetable proteins, especially in so-called reserve-proteins.

The author wishes to express his sincere thanks to Prof. Dr. U. Suzuki for his kind guidance.

ON THE SAPONIN OF ADZUKI BEAN.

By E. TAKAHASHI and K. SHIRAHAMA.

(Received Aug. 26th, 1926.)

Isolation and properties : Pulverized Adzuki-bean was extracted with 95% alcohol and the extraction was evaporated to dryness after the magnesium oxide had been added.

The residue was again extracted with alcohol and the saponin was precipitated with ether from the solution.

By the repeated precipitation as above, the pure saponin was obtained as a amorphous yellow powder.

The substance was soluble in water, alcohol or phenol and its aqueous solution foamed markedly on shaking and with concentrated sulphuric acid it gave a characteristic reddish purple coloration.

Elementary analysis gave the following results.

(1) 3.580mg. saponin gave	6.355mg. CO_2 2.250mg. H_2O
(2) 4 147mg. saponin gave	7.318mg. CO_2 2.540mg. H_2O

	(1)	(2)	Average
C.	48.41%	47.86%	48.13%
H	7.03%	6.82%	6.93%

Haemolysis : Haemolytic property was tested with red blood corpuscles of an ox. The washed corpuscles were mixed with physiological saline solution of saponin and kept one hour for 37°C. Haemolysis took place in 1.5% saponin solution.

Haemolysis : Pure saponin was boiled with 1 % sulphuric acid for 18 hours, by which the saponin hydrolysed into prosapogenin and a sugar, glucose.

By further hydrolysis with 6% sulphuric acid for 5 hours the prosapogenin was decomposed into sapogenin and arabinose.

By the estimation of these sugars, the quantitative ratio among sapogenin, arabinose and glucose was determined as 1 : 1 : 16.

The sapogenin was white amorphous powder insoluble in water, soluble in alcohole and phenol.

It was very stable for acid and alkali.

(1) 4.425mg. sample gave	10.820mg. CO_2 3.900mg. H_2O
(2) 2.925mg. sample gave	7.170mg. CO_2 3.050mg. H_2O

	(1)	(2)	Average
C	66.69%	66.85%	66.70%
H...	10 86%	11 02%	10.90%
O...	—	—	23.40%

From the above data and by the measuring of the freezing point depression of its phenol solution, its molecular formula was calculated as $C_{23}H_{45}O_6$.

So the molecular formula of Adzuki–saponin is determined as $C_{23}H_{45}O_6$. $C_5H_8O_4$. $16C_6H_{10}O_5$.

Discussion : Power and Salway isolated a kind of saponin from the root of a species of Adzuki, Phaseolus Multiflora (Pharm. Journ. p. 553, 1913) and gave the formula $C_{26}H_{44}O_4$ to the sapogenin, and $C_5 H_{44}O_{24}$ to the saponin. They isolated rhamnose by hydrolysis of the saponin and the ratio of sapogenin to rhamnose was determined as 1 : 4.

But the saponin isolated by us, appears to be quite different from that of Power and Salway. It in composed of a sapogenin, arabinose and glucose in the ratio 1 : 1 : 16. The difference is probably due to the difference in the species of the plant. It is also conceivable that substance in the roots undergoes change during the removal to the bean.

A NEW METHOD FOR QUANTITATIVE ESTIMATION OF STARCH BY ASPERGILLUS AMYLASE (TAKA-DIASTASE).

By Kokichi OSHIMA and Shin-ichi ITAYA.

(Received Sept. 2nd., 1926)

The procedure of the new method is as follows :-

Mix 1 gram of powdered sample with 80c.c. water in a flask of hard glass and cook for 10 min. at 100°C in an autoclave or for 1 hour in a boiling water bath. Add a mixture of 3.2c.c. M/6 citric acid and 6.7c.c. M/6 Na_2HPO_4 to keep the whole liquid at pH 5.2. Further add 10c.c. of 3.0% apueous solution of Taka-diastase (made by Sankyo & Co. or Park and Davis & Co.) or a strong enzymic preparation obtained from Aspergillus oryzae and 1 c.c. of toluol. Shake and close well, and keep it for 24 hours at 40°C. After that period, dilute the contents to 200c.c. with water and filter it through dry filter paper. With 20c.c. of the filtrate, determine the reducing activity by Bertrand's or other methods and calculate the reducing matter as glucose. Then multiply the quantity with 0.9 to obtain starch quantity. Of course it is necessary to subtract the reducing matter present in the sample before digestion and that produced by autolysis of Taka-diastase used.

The following results were obtained by the preliminary experiments and by application of the new method.

1. The optimum reaction of the amylo-saccharifying action of enzyme obtained from Aspergillus oryzae (Ahlb.) Cohn is pH 2.5.

2. One hour's heating of the enzyme in about neutral solution at a temperature below 45°C has no effect upon the amylase. At 50°C the activity is a little reduced, at 55°C it is reduced nearly half and above 65°C all the enzymic activity is lost. More prolonged heating has greater destructive action, but at a temperature lower than 40°C at almost neutral reaction, the amylase undergoes no change, even after one year, provided it is protected from other injurious action.

3. At pH 4.5 for one hour at room temperature, destruction of the amylase begins, and at pH 2.5 it is almost entirely destroyed; at pH 8.4 its activity also begins to decrease and at pH 10 almost all the activity is lost.

4. For heating the amylase solution, pH 6.4 is the most stable reaction.

5. The final decomposition product of starch by Asp. amylase is recognized to be glucose exclusively. However, if the quantity of the amylase used is less than a certain minimum, the glucose production is not complete even though the digesting period is prolonged.

6. For the cooking of starch samples, one hour in boiling water has almost same effect with cooking for 10 min. at 110°C in an autoclave.

7. Comparison of starch content in many kinds of cereals, roots and their products determined by the new method and by the decomposition with HCl is as follows :–

Starch Content in Dry Matter of Samples.

Sample	Moisture(%)	Starch (%)	
		By new method	By HCl method
Soluble starch	10	98.40	99.16
Dry potatoes	10	77.60	73.20
Dry sweet potatoes	15	71.78	75.39
Unpolished rice	10	71.30	72.40
Kaoliang	13	69.51	71.28
Mais	11	67.24	70.66
Starch residue	15	65.54	68.61
Barley	15	63.10	68.61
Italian millet	13	50.37	56.16
Oat	15	14.47	61.41
Wheat bran	13	28.35	46.13
Rice bran	11	16.96	30.84

The above results show that there is a big difference of starch content in oat, wheat bran and rice bran etc. These starch contents by the new method are much alike with that obtained by the method of malt diastase on which Sherman and others have reported.

8. The result of the alcohol fermentation of these samples corresponds well with the starch content determined by the new method.

9. This new method is more accurate than the HCl method and simpler and easier than the malt diastase method.

(Hokkaido Imperial University. August 1926)

THE NATURE OF THE ACIDITY OF MINERAL-SOIL.

(*Abstract.*)

By Shigeru OSUGI and Yoshio SANO.

(*Department of Agriculture, Kyoto Imperial University, Kyoto, Japan.*)

(Received Sept. 9th., 1926.)

1. The mineral-acid-soils are able to invert cane sugar to a remarkable extent and this inverting action is not due to the presence of any soluble acid substance in the soil but to the surface action of soil-particles; and from many experimental results, it is ascribed to the action of acid aluminium silicate in the soil.

2. The hydrogen ion concentration of the water extract of the soil is not high enough to explain the inverting action of soil.

It was proved that the higher proportion of soil to water (1/3–1/50) gives the higher hydrogen ion concentration in the extract, (0.05–0.5 as PH.) and that the extract of clay part ($<$0.01m.m.) has the higher concentration of hydrogen ions (0.2–1.5 as pH.) than that of coarser part ($<$0.5m.m.) of the same soil.

From these results, it is reasonably expected the hydrogen ion concentration in the absorbed water film around soil-particles; especially of the clay part, to be considerably higher than that in the surrounding liquid, and indeed, so high that it inverts cane sugar so distinctly as mentioned above.

3. Shaking the soil at a room temperature and at 90–100°C. accelerates the inversion reaction (5–40%) and this shows again that the reaction takes place mainly on the surface of soil-particles.

4. Grinding the soil-particles decreases the concentration of the hydrogen ions in the extract (about 0.1 as pH.) and diminishes the inversion reaction (8–35%), and repeated freezing and thawing the soil increases both, (0.02–0.28 as pH. and 6–34% of inversion) but the conductivity of the extract increases in both cases. (7–29% by grinding and 5–36% by freezing)

From the above results, it is concluded that the inversion reation occurs only on the surface of soil-particles having certain hydrogen ion concentration, and only on the insoluble acid or acid substance in soil.

5. The mineral-acid-soils, air-dried and even dried at 90–100°C can invert cane sugar in a glycerine solution (5% sugar disolved in glycerine free from water) and this shows again that the inversion reaction should occur

only on the surface of soil-particles.

6. When any weak alkali-solution is added to the soil suspension, one of the following changes in the conductance should take place according to the property of the suspension.

(1). When the suspension has no action upon the solution, the conductivity of the solution suffers no change.

(2). When the suspension adsorbs the base in the solution physically, the conductivity should decrease.

(3). When the ssupension is of acid-nature and combines chemically with base, the conductivity should increase.

And when a strong alkali-solution is added to the suspension of acid-nature, the conductivity should decrease.

A test was made with the acid-soil and ammonia solution, and the increase of the conductivity was noted (30-96%) but with sodium hydroxide solution, the distinct decrease was observed. (30-90%)

From these experiments, it is shown that the substance causing the acidity in the soil is of true acid-nature.

7. An experiment on the influence of heating the soil shows that the heating of two hours at 80°, 90°, and 100°C. causes no change upon the inverting action and the degree of acidity (hydrolytic and the exchange) but at 250°C., the former begins to diminish and at 550°C., it almost disappears.

Bouyoucos has recently carried out experiments on the effect of heating upon the physical properties of soil and reported that the heat of wetting, the unfree water and the plasticity of soil, begin to diminish at 230°C. and at 485°C., these properties are almost dissipated.

The results shows that the inverting action of the soil and the physical properties named above are closely correlated, and that the substance causing the inverting action of the soil is colloidal.

STUDIES ON PROTEINS IV.
ON THE PREPARATION OF RICE-GLUTELIN.

(Contribution No. 5 from the Laboratory of Nutritional Chemistry, Dept. of Agriculture, Kyoto Imperial University)

By

Kinsuke KONDO and Tunetomo HAYASHI.

(Received Sept 8th., 1926.)

A. In great probability it is impossible to prepare such a chemically pure single protein as we desire. This point was elucidated by Sörensen's experiments.(1) As is well known, we can find in polished rice besides other constituents four kinds of protein such as albumin, globulin, glutelin and prolamin. We may reasonably assume these proteins occur in the rice itself, as well as in the special protein fractions obtained from it, not as mixtures of proteins, but as their combinations, as Sörensen considers in regard to serum-globulins. Consequently even tho we might isolate a special protein fraction from the natural substance, this fraction could not consist of merely the desired single protein. We state herewith that it is a reasonable question to-day whether the fraction thus obtained is reproduceable or not. Presumably it would be secondary whether pure or not. However we may expect to prepare a well-defined and reproduceable protein. In studying the chemistry of proteins we shall be satisfied at present with such a protein as discussed above.

B. a. Rice-glutelin, as well as other kinds of proteins, capable of change in an alkali-solution, even tho this be not very strong. In preparing the rice-glutelin we may first remove the albumin and globulin from the rice-powder. For this purpose we extract the rice powder with a 10% NaCl solution 3 times continuously and then wash out completely till it contains no NaCl.

The residue is mixed with water and then with a 0.05n NaOH solution by means of dropping in such a manner that the concentration of NaOH will be under 0.025n. Then the rice-glutelin dissolves into the NaOH solution. This solution is clarified and freed from starch particles and other ingredients after repeated filtration. Into the clear solution thus obtained a certain amount of 0.05n acetic acid is dropped till the glutelin flocculates out as completely as possible. The glutelin precipitate is filtered and washed respectively and then redissolved and reflocculated. Such a procedure is repeated 3 times continuoulsy to make it free from other nitrogenous ingredients, and this was proved experimentally. In this whole treatment about one third of the nitrogenous substance in rice powder is lost.

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(1) Comptes-rendus du Lab. Carlsberg **12**. (1917)
Zeitschr, physiol. Chem. **103**. (1918)
Jour. Amer. Chem. Soc. **47**, 457. (1925)

B. b. We purified our rice-glutelin mineral substance and acetic acid by application of dialysis. The experiments show that the rice-glutelin becomes practically entirely free from any diffusible ingredient, such as mineral matter, in 6 or 7 days. Hence, we can believe that the protein thus purified contains practically no other ingredient. We call it Rice-glutelin No. 1.

B. c. We made our rice-glutelin into anhydrous state with the help of alcohol and ether and determined the amount of nitrogen and its distribution by the usual method.

B. d. It is proved that a 0.5n NaCl solution is a more reasonable solvent for removing globulin from the rice powder than a 10% NaCl solution, which is used commonly. Hereafter we shall prefer a 0.5n NaCl solution in the foregoing preparation of glutelin to any other.

(Sept. 5, 1925)

STUDIES ON PROTEINS V.

ON THE POINT OF OPTIMUM FLOCCULATION OF RICE-GLUTELIN.

(Contribution No. to from the Laboratory of Nutritional Chemistry, Dept. of Agriculture, Kyoto Imperial University).

By

Kinsuke KONDO and Tunetomo HAYASHI.

(Received Sept. 8th., 1926)

A. After Michaelis,(1) an iso-electric point of ampholyte difficult of solution, i. e. casein, falls on the point of an optimum flocculation.

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(1) Michaelis :- Biochem. Zeitschr. **47**, 250 u. 260. (1912)

The rice-glutelin is, like casein, an ampholyte difficult of solution. Hence the rice-glutelin must flocculate at a maximum if the reaction of the solution is to become an iso-electric point of the rice-glutelin. But even if our rice-glutelin successively flocculates and precipitates at a maximum in appearance a part of the protein remains in the dissolved from. Therefore we may reach a conclusion as to whether the reaction of the solution is apart from the iso-electric point of our rice-glutelin or whether the so-called optimum or maximum flocculation does not mean the perfect flocculation. We will in the present work examine the point of flocculation of our rice-glutelin and study its condition in the state of optimum flocculation.

B. We examined the point of optimum flocculation of Rice-glutelin No. 1 in acetate-acetic acid mixtures. We find that this protein can flocculate and precipitate at a maximum in the diluted sodium acetate solution, whose reaction is near absolute neutrality.

And our experimental results show that the more diluted the sodium acetate solution becomes, the farther the reaction goes towards the iso-electric reaction of this protein.

But the rice-glutelin can not completely flocculate even at the point of optimum flocculation, and the latter is changeable according to the presence of any salt in the solution. This was elucidated by means of acetate solutions and Sörensen's phosphate mixture. We find also that the reaction of the solution, in which the protein can flocculate at a maximum, changes after the addition of the protein.

C. We searched for the cause of this in the difference of the protein-ionizing powers of the ions derived from the salt, beside the hydrogen ion activity. And many salts have such a property. Hence the protein can not completely take form of $[R<\begin{smallmatrix}NH_2\\COOH\end{smallmatrix}]$ in the presence of salt in the solution. In other words, the protein can not behave as an iso-electric ion in the presence of salt in the solution.

Applying these facts and considerations to the theory on the iso-electric point, we conclude it is impossible to determine the iso-electric point of a protein difficult of solution, such as casein and rice-glutelin, by the usual method. Hence we define the point, determined by this last, as an apparent iso-electric point and differenciate it from a theoretical one. But for a protein such as native albumin, whose iso-electric reaction is independent of the presence of salt, the apparent and theoretical iso-electric points are identical with each other. (August 8, 1926)

UEBER DIE REINIGUNG DER AMYLASE AUS ASPERGILLUS ORYZAE.

von Sukeharu NISHIMURA.

(Ausgegangen am 15. Sept., 1926)

EINLEITUNG:

Seit der Entdeckung der Malzamylase bis auf den heutigen Tag war das Bestreben zahlreicher Forscher darauf gerichtet, viele enzymatisch unwirksame Begleitstoffe durch chemische und physikalische Methoden mehr und mehr zu entfernen, das Enzym in reinerem Zustand zu erhalten und schliesslich es auf seine chemische Natur hin zu untersuchen.

Lange Zeit galt als der gangbarste Weg zur Gewinnung von Malzamylase in Substanz die Lintner'sche Methode,[1] nach welcher die enzymhaltigen Malzauszüge im Verhältnis 1 : 2 mit Alkohol gefällt werden. Die beliebte Methode, die bisher hochwertigsten Malzamylasepräparate zu gewinnen, ist die Sherman und Schlesinger'sche[2] fraktionierte Fällung mit Alkohol oder Aceton. Sie dialysieren die Enzymlösung bei niedrigen Temperaturen und fällen den Dialysierrückstand fraktioniert mit Alkohol. Die zwischen 50 und 65 proz. Alkoholgehalt ausfallende Fraktion hat die kräftigsten enzymatischen Eigenschaften. Sie gelangen auf diesem Wege von dem Fermentativ-Vermögen der Würze Fz=1 gewöhnlich zu Amylasepräparaten vom Fermentativvermögen Fz=ca. 30. Dieser Wert von Fz=30 wird allerdings nur unter günstigen Umständen erreicht, man kann mit Fz=10—15 schon zufrieden sein. Neuerdings wird auch die Reinigungsmethode mit Hilfe der aufeinanderfolgenden Elektrodialyse und Elektroosmose von Fricke und Kaja[3] verwendet; diese Forscher erhielten eine Steigerung der enzymatischen Kraft auf den 5 fachen Betrag gegenüber dem Ausgangsmaterial.

Ein jedes bis auf den heutigen Tag dargestellte reinere Malzamylasepräparat enthält in überwiegender Menge Eiweiss. Fricke und Kaja behaupten eiweissfreie oder wenigstens eiweissarme Lösungen in Händen gehabt zu haben.

In den letzten Jahren ist nun von R. Willstätter[4] und seinen Schülern ein neues Verfahren der Enzymreinigung ausgebaut worden, das nach seinen Leistungen alle bisherigen Methoden weit übertrifft und bei jenen Enzymen,

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1) Lintner: Journ. f. prakt. Chemie (2), 34, 378 (1886).

2) Sherman u. Schlesinger: Journ. Americ. Chem. Soc. 35, 1617 (1913).

3) Fricke unp Kaja: Berichte 57, 310; 765 (1924).

4) R. Willstätter: Zeitschr. f. phys. Chem., 123, 45 (1922), siehe auch Liebigs Annalen, 1918 und sf.

bei welchen es systematisch Anwendung fand, wie beim Invertin und der Peroxydase, zu Präparaten von bisher unerreichter und ungeahnter Reinheit führte. Der Grundgedanke dieses Verfahrens ist der, aus der unter Umständen nach einem der älteren Verfahren vorbehandelten Enzymlösung das Enzym mit etlichen Begleitstoffen an ein geeignetes Adsorptionsmittel zu adsorbieren. Ein grosser Teil von Verunreinigungen bleibt dabei in der Lösung. Aus dem Adsorbat wird sodann durch ein geeignetes Lösungsmittel das Enzym herauseluiert, wobei wieder nur ein Teil der Begleitstoffe mit dem Enzym in die Elution übergeht, ein anderer dagegen am Adsorptionsmittel verbleibt. Die Elution kann sodann einer weiteren Adsorption mit dem gleichen oder einem anderen Adsorbens unterworfen werden. Eine folgende Elution liefert dann wieder ein reineres Enzympräparat. Diese Operationen der Adsorption und Elution können unter Umständen vielmals hintereinander wiederholt werden.

In der jüngsten Zeit haben H. Lüers und E. Sellner⁵⁾ diese Reinigungsmethode an der Malzamylase zur Anwendung gebracht und gute Erfolge erzielt. Es gelang ihnen durch Aneinanderreihung von zwei Tonerdeadsorptionen und zwei Elutionen, vom Malzauszug ausgehend, den Reinheitsgrad des Enzyms von Fz=ca. 1 auf Fz=ca. 25—30 zu erhöhen. Weiter ausgehend vom Amylasepräparat von Fz=ca. 10, das nach der Methode von Sherman und Schlesinger gewonnen war, gelangen sie durch Vornahme zweier Adsorptionen und Elutionen zu Fz=ca. 45—60, in seltenen Fällen sogar zu Fz=93. Die Analyse ihrer reinsten Präparate ergab einen Proteingehalt von 74% und einen Kohlehydratgehalt von 28.07% der phosphorsalzfreien Substanz. Die Gegenwart von Pentosen, liess sich durch die Furfurolreaktion nachweisen. Also scheint ein bestimmter Eiweisskörper zum Enzym in näherer Beziehung zu stehen.

So einfach im Prinzip dieses Verfachren erscheint, so schwierig ist seine praktische Durchführung; eine Unmenge von Vorarbeiten müss geleistet werden, bis man den richtigen Weg, das geeignete Adsorptions-und Elutionsmittel, die zweckmässigen gegenseitigen Mengenverhältnisse, die günstigsten Bedingungen usw. gefunden hat. Glaubt man sich am Ziel, so zerrinnt nicht selten der Erfolg mühevoller Arbeit in nichts, da die Enzym mit fortschreitendem Reinheitsgrad immer labiler und empfindlicher gegen äussere Einflüsse werden.

Ueber die Enzym von Koji, einem aus Aspergillus oryzae, der auf gedämpftem Reis gezüchtet wurde, hergestellten Material, das in Japan schon tausend Jahre hindurch in verschiedenen Gebieten grosse Anwendung findet, besonders über die Enzym der Takadiastase, wie sie in dem von Takamine fabrizierten Präparat vorkommen und wegen ihres Gehaltes an mannigfachen

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5) H. Lüers und E. Sellner: Wochenschr. f. Brau. 43. 97 (1925)

abbauenden Enzymen insbesondere ihrer höheren diastatischen Wirkung auch für medizinisch-klinische Zwecke verwendet wird, sind viele frühere Arbeiten vorhanden. In erster Linie war das amylolytische Enzym schon mehrfach Gegenstand eingehender Untersuchung, besonders in den Arbeiten von Atkinson,[6] Kellner, Mori und Nagaoka,[7] Stone und Wright,[8] Saito[9] und Takamine.[10] Man verdankt Saito[11] eine Untersuchung über den Einfluss verschiedener Nährböden auf die Amylasebildung des Aspergillus oryzae.

J. Wohlgemuth[12] untersuchte orientierend die verschiedenen Enzyme der Takadiastase, nämlich die amylolytische, proteolytische (darunter tryptische, labende, ereptische peptolytische) Wirkung und die Lipase, er konnte nur die peptolytische Wirkung nicht nachweisen. Die Verwendung von Stärke als Substrat für die Amylasewirkung haben Sherman und Baker[13] eingehend besprochen. Auch über die Amylasewirkung haben Sherman und Punnet,[14] Sherman und Thomas,[15] Waksman,[16] Takamine,[17] über die proteolytische Wirkung Okada,[18] Szanto,[19] über die Saccharase Bertrand und Rosenblat,[20] über die Katalase Neidig,[21] und noch viele Forscher gearbeitet. Die Resultate aber waren in manchen Punkten widersprechend, entweder fehlten exakte Methoden der enzymatischen Wirksamkeitsbestimmungen, oder es wurden wenig zuverlässige Methoden in Anwendung gebracht.

Neuerdings haben C. Neuberg[22] und seine Mitarbeiter die verschiedenen Enzyme der Takadiastase untersucht und mit Hilfe der exakten Methoden der neueren Enzymchemie neue darin gefunden und die zur Diskussion gestandenen Probleme klar gestellt.

Ueber die Reinigung der Pilzamylase wurde bisher noch wenig gearbeitet. Die Takadiastase ist gewöhnlich nur ein durch einfache Alkoholfällung des Auszuges von Koji gewonnenes Material, also ein unreines Präparat, welches meistens unter Fz=ca. 2 hat. Die Takadiastase des Handels kann nach

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6) Atkinson: Transact. of the Chem. Soc. London (1881).
7) Kellner, Mori und Nagaoka: Ztschr. f. phys. Chem. 14. 297 (1890).
8) Stone und Wright: Journ. Americ. Chem. Soc. 20. 637 (1898)
9) Saito: Centralbl. f. Bakt. (2) 17. Heft 1—7 (1906).
10) Takamine: Journ. soc. chem. Ind. 17. 118 (1898).
11) Saito: Wochenschr. f. Brau. 27. 181 (1910).
12) Wohlgemuth: Biochem. Zeitschr. 39. 324 (1912).
13) Sherman und Baker: Journ. Americ. Chem. Soc. 38. 1885 (1916).
14) Sherman und Punnet: do. 38. 1877 (1916).
15) Sherman und Thomas: do. 41 (1919).
16) Waksman: do. 42 (1920).
17) Takamine: do. 42 (1920).
18) Okada: Biochem. Journ. 10. 130.
19) Szanto: Biochem. Zeitschr. 43. 31.
20) Bertrand und Rosenblat: C. v. d. l'Acad. des Sciences 156. 261.
21) Neidig: Journ. Americ. Chem. Soc. 36 (1914). Ueber die Takadiastase siehe auch Euler. Chemie der Enzyme II. 127.
22) Neuberg und Kurono: Biochem. Zeitschr. 140. 295. (1923) u. sf. Siehe auch Heuss: Tagesztg. f. Brau. 560. (1925)

Sherman und Tauberg[23] bis auf das 30fache der ursprünglichen Wirksamkeit angereichert werden durch Extraktion mit Wasser, Ausfällen mit Ammoniumsulfat, Dialysieren und fraktionierte Fällung mit Alkohol. Die gereinigten Produkte zeigen noch folgende Proteinreaktion; Millon, Xanthoprotein, Tryptophan-, Biuretreaktion. Charakteristisch für die diastatischen Präparate aus Aspergillus oryzae ist das hohe Verhältnis der stärke- verflüssigenden und verzuckernden Fähigkeit.

Ein nach besonderem Verfahren von Takamine dergestellter Extrakt, Polyzime genannt, ist von Takamine und Oshima[24] studiert worden. Die Verzuckernde Fähigkeit geben diese Forscher folgendermassen an:

	21°	50°
Lintner-Einheiten	43	150
oder als Sf.	1.1	3.9

Die chemische Zusammensetzung ist nach Takamine und Oshima:

Wasser 87.5% Feste Substanzen 12.5% Asche 1.5%

Nachdem ich[25] nun an der Takadiastase die Reinigungsmethode nach Willstätter zur Trennung der verschiedenen in ihr vorkommenden Enzyme ziemlich viel untersucht habe, stellte ich mir die Aufgabe, an der Amylase des Pilzes, nicht von der Takadiastase sondern vom Pilz ausgehend, systematisch den Weg der Adsorptionsreinigung zu gehen, wie er von Willstätter an der Peroxydase und dem Invertin in vorbildlicher Weise gezeigt und geebnet, und von Lüers und Sellner an der Amylase des Malzes verwendet wurde. An den reinsten Präparaten wollte ich dann versuchen, den Fortschritt der Reinigung analytisch-chemisch, insbesondere im Vergleich mit den reinsten Malzamylase-Präparaten festzustellen. Auch sollte dabei beachtet werden, möglichst ohne grosse Verluste an Enzym zum Ziel zu kommen.

EXPERIMENTELLER TEIL.

I. Allgemeines über die Versuchsmethodik.

Obwohl die Wirksamkeitsbestimmungsbedingungen der Amylase schon in meiner ersten Arbeit erwähnt wurden, müssen sie hier nochmals wiederholt werden, weil eine exakte Bestimmung der enzymatischen Wirksamkeit die Grundbedingung aller Arbeiten besonders auf dem Fermentgebiet ist.

BEDINGUNGEN UEBER DIE WIRKSAMKEITSBESTIMMUNG DER AMYLASE.

Die Wirksamkeitsprüfung der Amylase beruht auf der quantitativen Messung der Menge Maltose, welche des Enzym unter genau festgelegten Bedingungen aus seinem spezifischen Substrat, der Stärke, zu bilden vermag.

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[23] Sherman und Tauberg: Journ. Americ. Chem. Soc. 38. 1638 (1916).

[24] Takamine und Oshima: Journ. Americ. Chem. Soc. 42. 1261. (1920)

[25] S. Nishimura: Chem. d. Zelle u. Gewebe. 12. 201 (1925).

Als Substrat Verwendete ich zu allen Versuchen lösliche Stärke, die von Kahlbaum bezogen wurde. Bei sämtlichen Amylasewirksamkeitsbestimmungen verwendete ich die Stärkelösungen, 3 prozentig iu Bezug auf Stärketrockensubstanz. 3g. Stärketrockensubstanz wurden mit destilliertem Wasser angeschlämmt und die milchähnliche Lösung in ca. 60 ccm, in einem 100 ccm-Kölbchen in lebhaftem Sieden befindliches Wasser eingegossen und darauf noch 3 Minuten weiter gekocht. Das Abkühlen erfolgt unter Verschluss, um eine Hautbildung zu vermeiden.

Zur Festlegung der optimalen Wasserstoffionenkonzentration der Amylasewirkung setzte ich der Stärkelösung jeweils 6.4 ccm $\frac{n}{10}$ Essigsäure und 10 ccm $\frac{n}{10}$ Natriumazetatlösung zu; dieses Puffergemisch entspricht einer $(H') = 1{,}3.\ 10^{-5}$.

Dieser so vorbereiteten Stärkelösung wurde dann die abgemessene Enzymlösung (meistens 5 ccm) zugesetzt, sofort rasch auf 100 ccm mit Wasser aufgefüllt, umgeschüttelt und die Zeit festgestellt. Die Einwirkung der Amylase auf die Stärke liess man immer bei der konstant gehaltenen Temperatur von 20°C. vor sich gehen.

Nach bestimmter Zeit (immer 30 Min.) entnimmt man dem Reaktionsgemisch Proben von 15 ccm und lässt sie in ein 100 ccm Kölbchen, in dem 30 ccm Fehling'sche Lösung sich befinden, einfliessen, wodurch augenblicklich die Vernichtung des Enzyms erfolgt. Die Menge der gebildeten Maltose wurde auf massanalytischem Wege nach Kjeldahl-Bertrand bestimmt. Das Reduktionsvermögen der Stärke-und der Enzymlösung wurde in einem blinden Versuche ermittelt und in Abzug gebracht.

Das zu prüfende Enzymmaterial war meist schon in Lösung vorhanden. In diesem Falle brauchte dann nur noch eine Trockensubstanzbestimmung durch Trocknen eines gemessenen Volumens im Vakuum vorgenommen zu werden.

BERECHNUNG DES VERZUCKERUNGSVERMÖGENS DES AMYLASEPRÄPARATES.

Die Reaktionsgeschwindigkeitskonstante K, die als Mass für die Intensität des Abbaues angesehen werden kann, ergibt sich aus der Gleichung.[26]

$$K = \frac{1}{t.\ 0.4343} \cdot \log \frac{a}{a-x}$$

dabei ist

t ··· ··· ··· ··· die Wirkungszeit (30 Min.)
x ··· ··· ··· ··· die Maltosemenge, welche nach der Zeit t gebildet wird.
a ··· ··· ··· ··· spaltbare Substanzmenge oder Anfangskonzentration.

Da der Grenzabbau der Stärke bei 75% Maltose liegt, wird $a = 3.\ 0.75 = 2.25$ in Rechnung gesetzt.

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[26] Lüers und Sellner, loc. cit.

Mit Hilfe dieses K–Wertes lässt sich dann das Verzuckerungs–vermögen des Amylasepräparates Fz folgendermassen ausdrücken:

$$\mathrm{Fz} = \frac{\mathrm{K.}\ \mathrm{g\ Stärke}}{\mathrm{g\ Trockensubstanz\ des\ Enzympräparates}}$$

Diese Fz–Werte sind miteinander vergleichbar. Steigt Fz im Verlaufe einer Operation, die man mit dem Enzyme vornimmt, an, so heisst das, das Enzym hat an Wirksamkeit zugenommen, die Trockensubstanz ist an Enzym reicher, an Begleitstoffen ärmer geworden. Die Fz=Werte setzen uns also in den Stand, über den Erfolg der vorgenommenen Enzymreinigungsmethoden quantitative Schlüsse zu ziehen.

TROCKENSUBSTANZBESTIMMUNG IN DEN PHOSPHATELUTIONEN.

Die Phosphatlösung hat sich unter den bisher untersuchten Elutionsmitteln am besten bewährt. Aber ein Nachteil davon ist der, dass die Trockensubstanz der Elutionen grosse Phosphatmengen enthält, die eine exakte Trockensubstanzermittlung, wie sie für die Fz-Berechnungen nötig sind, sehr erschweren. Es geht auch nicht an, einfach die berechnete Phosphatmenge, unter Berücksichtigung des Kristallwasserverlustes beim Trocknen vom ermittelten Trockensubstanzgewicht zu subtrahieren, denn ein Teil des Phosphates bleibt an der Tonerde haften. Deshalb wird die gewogene Trockensubstanzmenge nach Lösen in Salpetersäure nach von Lorenz quantitativ auf Phosphorsäure untersucht.

Aus dem P_2O_5–Gehalt lässt sich dann die Menge des Phosphatgemisches von Ph=8.0, die bei meinen Versuchen immer verwendet wurde und aus 9.4 ccm $\frac{m}{15}$ Na_2HPO_4 + 0.6 ccm $\frac{m}{15}$ KH_2PO_4 besteht, nach folgendem Ansatz berechnen:

$$\begin{array}{lcccc} & \text{9,4 mol.} & + \ \text{0.6mol.} & \text{entsprechen} & \text{5mol.} \\ & Na_2HPO_4 & KH_2PO_4 & & P_2O_5 \\ & \text{(wasserfrei)} & & & \\ = & \text{1335,67 g.} & + \ \text{81.684g} & & \\ = & & \text{1416,88 g} & & = \text{710,4 g} \end{array}$$

Die durch die Analyse ermittelte P_2O_5–Menge, multipliziert mit $\frac{1416.88}{710.4} = 1.994$ liefert die von der Trockensubstanz zu subtrahierende Phosphatmenge.

II. Gewinnung der Amylase.

Zuerst wurde geprüft, auf welchem Nährboden der Pilz am besten die Amylase zu bilden vermag, um damit ein gutes Ausgangsmaterial des Enzyms zu bekommen. Als Nährmaterialien wurden Würze, Reiskleister, Reis–Weizenkleiekleister, gedämpfter Reis, gedämpfte Weizenkleie folgendermassen geprüft;

1. 8%ige Würze. Auf die Würze, die in grossen Glasschalen sich befand und sterilisiert war, wurden die Sporen des Pilzes, die in der wissenschaftlichen Station für Brauerei in München aufbewahrt waren und auf Würzegelatinplatte

gezüchtet wurden, ausgesät und bei ca. 30°C. im Thermostat aufbewahrt. Nach 4—5 Tagen wurde das Myzel, das auf der Würze sich als weisser Rasen entwickelte, aus der Würze herausgenommen, mit wenig Wasser gewaschen, dann bei ca. 40°C. im Faust-Heim-Trockenapparat getrocknet und pulvensiert.

Eine bestimmte Menge des Präparates wurde mit Wasser ohne oder unter Znsatz von Toluol mit Sand zerrieben und das Filtrat, wie schon erwähnt, auf 3%iger gepufferter Stärkelösung einwirken gelassen. Der nach 30 Min. bei 20°C. gebildete Zucker wurde bestimmt.

2. 10—20% Reis wird mit Wasser zu Kleister verkocht, in die Glasschale hineingegossen, darauf werden die Sporen ausgesät. Weiter wurde wie bei 1) verfahren.

3. 20% Reis und Weizenkleiegemisch wurde mit Wasser zu Kleister verkocht und weiter wie oben behandelt.

4. Auf gedämpftem Reis entwickeltes Myzel wurde mit dem Reis zusammen mit Wasser und Sand zerrieben und klar filtriert.

5. In gedämpfter Weizenkleie entwickeltes Myzel wurde, wie später eingehend erwähnt wird, behandelt.

Als Beispiel von mehreren Versuchen sei Folgendes angeführt.

	15 ccm Verzuckerungsgemisch verbrauchten Permanganat nach 30′. (ccm)	Trockensubstanz (in 5 ccm)
1	4.1	0.2 g.
2	5.7	0.2 g.
3	8.2	0.2 g.
4	5.8	0.244 g.
5	13.8	0.0925g.

Also wie Saito[27] und Takamine[28] berichtet haben, wird die Amylase am meisten beim Wachstum auf gedämpfter Weizenkleie gebildet. Infolgedessen wurde, um die Amylase zu gewinnen, zu den weiteren Versuchen stets Weizenkleie als Nährmaterial verwendet.

Je 100g. Handels-Weizen-Kleie werden mit ca. 70 ccm Wasser befeuchtet, in einen Sack gebracht und in einem Topf eine Stunde gedämpft. Nachdem die Masse in der Glasschale sich abgekühlt hatte, wurde sie mit Pilzsporen gut geimpft und bei ca. 30°C. im Thermostat 3—4 Tage gehalten, bis das weisse Myzel gut wächst. Die ganze Menge wird mit Sand und wenig Wasser zerrieben, weiter ca. 800 ccm Wasser zugegeben, umgerührt und nach 1 Stunde durch Faltenfilter filtriert. Man erhält eine trübe Lösung. Um diese Trübung zu beseitigen, wird eine ziemlich grosse Menge Kieselguhr in die Lösung gegeben, gut gemischt und dann durch eine Nutsche klar filtriert.

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27) Saito: Wochenschr. für Brau. 27. 181 (1910).

28) Takamine: Journ. Ind. und Engin. Chem. 6. 824 (1914).

Dabei adsorbiert die Kieselguhr keine Amylase. Es wird dadurch ca. 650—700 ccm klarer Auszug gewonnen, der im Eisschrank zur Untersuchung aufbewahrt wird. Wegen des leichten Wachsens fremder Bakterien sind die Auszüge nur kurze Zeit haltbar, deshalb muss man öfter mit Kieselguhr filtrieren. Die Untersuchungen werden dadurch erschwert, dass die Beschaffenheit der Auszüge mehr oder weniger sich verändert, obwohl die Amylasewirksamkeit sich wenig vermindert.

Das Verzuckerungsvermögen Fz der Auszüge schwankt zwischen 0.2—0.4, ihr PH war ca. 6—5.5. Es ist also das Verzuckerungsvermögen der Auszüge kleiner als das des Malzes, das gewöhnlich Fz=1 ist:

Als Beispiel:

angew. ccm der Auszüge	g Maltose nach 30 Min.	Tr. Sub. in 5 ccm.	Fz.
5	0.7366	0.0742	0.38
5	0.6813	0.0961	0.28
5	0.5767	0.0684	0.33
5	0.4180	0.0788	0.196
5	0.7207	0.1260	0.23

Um weiter zu prüfen, ob mit anderen Lösungen als Wasser die Amylase noch besser herausgelöst werden kann, wurden folgende Versuche angesetzt:

10g. Kleiematerial, in dem der Pilz sich kräftig entwickelt hatte, wurden mit 100 ccm verschiedener Lösungen extrahiert und mit Kieselguhr klar filtriert. Zur Bestimmung des Verzuckerungsvermögens wurden 5 ccm davon verwendet.

		15 ccm Verzuckerungsgemisch verbrauchten Permanganats nach 30 Min.	Tr. Subs. in 10 ccm.
1.	20%ige Alkohollösung, 1 Std.	13.4 ccm	0 126 g.
2.	10%ige Kochsalzlösung, 1 Std.	12.7 ccm	—
3.	Mit Aether und Sand zerrieben, nach dem Vertreiben von Aether mit 100 ccm Wasser digeriert, 1 Std.	14.0 ccm	0.137 g.
4.	Mit Sand und Wasser, wie oben geschrieben, 1 Std.	13.5 ccm	0.122 g.
5.	do., 3 Stdn.	13.8 ccm	0.127 g.

Die Unterschiede sind nicht gross, doch zeigt sich, dass wenn die Amylase mehr herausgelöst wird, die Menge der Trockensubstanz auch steigt. Deshalb wurde zum Extrahieren immer nur Wasser verwendet.

III. Vorreinigungsversuche.

Kaolin und Kieselguhr können in dem Auszug von PH ca. 6 die Amylase nicht adsorbieren. Wenn sie aber andere Substanzen adsorbieren können, so kann man damit den Auszug vorreinigen. Um dies zu prüfen, wurden folgende Versuche angesetzt.

0.5 Kaolin oder Kieselguhr wurden in 10 ccm Auszug gegeben und gut gemischt. Nach den Zentrifugieren wurden 5 ccm des Filtrats vom Adsorbat getrocknet und gewogen.

5 ccm Auszug enthielten 0.100g. Trockensubstanz.
5 ccm der Filtrate enthielten 0.097—0.099g. Tr. Sub.

Also wird die Trockensubstanz auch durch diese Voradsorption fast nicht vermindert. Wenn man den Auszug mit NaOH oder HCl versetzt bis schwache Trübung auftritt, dann wird wohl die Trockensubstanz vermindert, jedoch wird auch ein Teil der Amylase vernichtet.

Wenn kleine Mengen Tonerde wenig Amylase, aber verhältnismässig mehr andere Substanz adsorbieren dann könnte man damit den Auszug vorreinigen. Um dies zu prüfen, wurde 1 ccm bezw. 2 ccm Tonerde-Sol mit 10 ccm Auszug versetzt. Nach dem Zentrifugieren wurden Trockensubstanz und Verzuckerungskraft der Filtrate in je 5 ccm bestimmt.

	angew. ccm	Tr. Subst. in 5 ccm	15 ccm Verzuckerungsgemisch verbrauchten Permanganats nach 30′.
Auszug	5	0.0925 g.	14.2 ccm
mit 1 ccm Tonerde	5	0.0918 g.	13.4 ccm
mit 2 ccm Tonerde	5	0.0873 g.	11 0 ccm

Je mehr also Trockensubstanz durch Tonerde adsorbiert wird, desto mehr wird auch das Enzymmaterial adsorbiert. Mit so kleinen Mengen Tonerde, welche das Enzym nicht mehr adsorbieren, ist keine Entfernung nennenswerter Mengen Trockensubstanz möglich. Man kann also weder mit Tonerde noch mit Kaolin und Kieselguhr, wie diese Versuche zeigen, den Auszug vorreinigen.

IV. Reinigungsversuche.

a) Versuche durch Tonerdeadsorption.

Nach den bisherigen Erfahrungen, die über die Adsorptionsreinigung von Amylase vorliegen, hat sich als Adsorptionsmittel kolloide Tonerde, als Elutionsmittel die Phosphatlösung ausgezeichnet bewährt. Deshalb beschränkte auch ich mich bei den folgenden Versuche auf Tonerde und Phosphat.

Die Darstellung der kolloiden Tonerde und die Arbeitsmethoden der Adsorption und Elution sind schon in meiner ersten Arbeit[29] genau angegeben. Durch die augwählende Adsorption blieben tote Begleitstoffe des Enzyms in der Ausgangslösung zurück, durch die selektive Elution ging das Enzym wiederum reiner vom Adsorbat in die Lösung über. Die Reinigungswirkung suchte ich dadurch zu verstärken, dass ich mehrere solcher Adsorptionen und Elutionen aufeinanderfolgen liess. 100 ccm der Tonerdesuspension, die stets hierbei verwendet wurde, enthielten 0.955g. Al_2O_3.

In mehreren Versuchen, von einer Ausgangsflüssigkeit von Fz = 0.2—0.4 ausgehend, gelang es durch einmalige Adsorption und Elution zu Fz = ca. 2—3, durch zweimalige Adsorption und Elution zu Fz = ca. 5—6 zu kommen.

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[29] Nishimura: loc. cit.

Während die erste Elution schwach gelblich gefärbt war, erschien die zweite durchwegs als völlig farblos. Durch dreimalige Adsorption und Elution nur mit Tonerde und Phosphat konnte der Wert nicht bedeutend gesteigert werden.

Man kann den Auszug fast ohne Verlust von Amylasewirksamkeit im Vakuum konzentrieren. 300 ccm Auszug wurden im Claisenkolben, wie bei der Vakuumdestillation, unter Minderdruck von 13—14 mm. bei 40—45°C. ca. in 2 Stunden ungefähr zur Hälfte eingeengt.

	angew. ccm	15 ccm Verzuckerungsgemisch verbrauchten Permanganats.
Ausgangsauszug	5	10.8
Konz. Auszug	5	20.8

	Tr. Subst. in 5 ccm.
Ausgangsauszug	0.064 g.
Konz. Auszug	0.132 g.

Das Eindampfen hat also die Amylase nicht geschädigt. Die Adsorptionen und Elutionen an diesen konzentrierten Auszügen sind etwas besser als am Ausgangsauszug gegangen:

Es gelang durch die einmalige Reinigung zu Fz=ca. 3—5, durch die zweimalige Reinigung zu Fz=ca. 7—9 zu kommen.

Als Beispiel diene folgender Versuch:

20 ccm Auszug wurde mit 20 ccm Tonerdesuspension (100 ccm enthielten 0.955g. Al_2O_3) versetzt, nach 1/4 Stunde zentrifugiert, das Adsorbat einmal mit 40 ccm Wasser in der Zentrifuge gewaschen, dann mit 12 cm Phosphatgemisch + 8 ccm Wasser von PH=8.0 1/2 Stunde lang unter Eiskühlung und Turbinierung eluiert. 20 ccm der Elution wurden mit 10 ccm Tonerdesuspension, welche 4.8 ccm $\frac{n}{10}$ HCl zwecks Einstellung des Gemisches auf PH=5.0 enthielt, zum zweitenmal adsorbiert und nach der gleichen Behandlung, wie bei der ersten Adsorption mit 4 ccm Phosphatgemisch + 16 ccm Wasser in gleicher Weise eluiert. Eine grosse Menge Auszug wird auf diese Weise in kleinen Portionen gereinigt.

Nachstehend die Belege:

Enzymmaterial.	angew. ccm	g Maltose in 100 ccm nach 30 Min.	K	g Tr. Sub. in 100 ccm Enzymlösg.	Fz.
Auszug	5	0.836	0.01546	3.640	0.1911
Filtrat von Adsorbat	5	—			
1. Elution	5	0.710	0.01264	0.1275	4.46
2. Elution	5	0.555	0.009448	0.0514	8.27

Das Adsorptionsvermögen des Al-Hydroxydes hängt im wesentlichen von seiner Darstellungsweise ab. Veranlasst durch diese Tatsache haben in neuester Zeit R. Willstätter und H. Kraut[30] eingehende Untersuchungen hierüber angestellt.

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30) Willstätter und Kraut: Berichte 56. 149. 1117. (1923).

Wie früher schon erwähnt wurde und folgende Zahlen auch beweisen, zeigt sich bezüglich des Einflusses der Wasserstoffionenkonzentration auf die Adsorption des Enzyms an Tonerde, dass schwach-saure Reaktion von P_H etwa 5 sie am meisten begünstigt. Neutrale oder schwach alkalische Reaktion setzt sie dagegen bedeutend herab.

Material:	angew. ccm	Zusatz.	adsorbiert bei P_H	g Maltose nach 30′.
Filtrat von Adsorbat	5	0	5.8	0.0375
	5	1 ccm $\frac{n}{10}$ HCl	5.0	0.0331
	5	1 ccm $\frac{n}{10}$ NaOH	8.0	0.0705

Der vorwiegend saure Komplex Enzym-Begleitstoffe wird also von der basischen Tonerdesuspension sehr gut adsorbiert. Stumpft man die Acidität ab, so verringert man den elektronegativen Charakter des Enzymkomplexes, was Verminderung der Adsorption an die positive Tonerde zur Folge hat.

Für die Reinigung des Enzyms von Begleitstoffen ist die Selektivität der Adsorption von grosser Bedeutung. Man will möglichst viel vom Enzym und möglichst wenig von unerwünschten Begleitstoffen im Adsorbat vorhanden haben. Deshalb muss man die Mengenverhältnisse so wählen, dass mit möglichst wenig Tonerde möglichst viel Enzym adsorbiert und mit möglichst wenig Phosphatgemisch möglichst viel Enzym eluiert werden.

Sämtliche Versuche sind selbstverständlich immer unter Eiskühlung vorgenommen. Um die Verzuckerungskräfte leicht vergleichen zu können, wurden alle Elutionsvolumina gleich gross wie die Anfangsvolumina gewählt.

b) Versuche durch Fällung des Enzyms mittels Aceton.

Die fraktionierte Fällung des Enzyms nach der Methode von Sherman und Schlesinger erfolgte hierbei mit Aceton ohne vorangehende Dialyse. Um richtige Mengenverhältnis zu bekommen, wurden folgende Versuche durchgeführt.

Gemessene Mengen Aceton und Auszüge werden vermischt (immer unter Eiskühlung), zentrifugiert und zur auszentrifugierten Lösung wieder eine bestimmte Menge Aceton zugegeben und neuerdings zentrifugiert. Die beiden Niederschläge wurden mit Aceton und Aether gewaschen und im Vakuum getrocknet. Diese Präparate wurden zur Verzuckerungsbestimmung verwendet.

Das Mengenverhältnis Aceton: Auszug hängt von der Konzentration des Auszuges ab. Je mehr die Konzentration zunimmt, desto weniger muss man Aceton zugeben.

Folgende Tabelle zeigt als Beispiel das Ergebnis:

	20 ccm Auszüge		1. Zugabe		2. Zugabe			
	Tr. Sub. g.	g. Maltose nach 30′.	Aceton menge ccm.	g. Maltose nach 30′.	Acetonmenge ccm	g. Maltose nach 30′.	Tr. Sub. g.	Fz.
1.	0.315	1.664	20	0.4393				
2.	0.936	6.587	20	1.4187				

				0.5226	15	4.909	0.0940	1.87
					10	3.856	0.0543	2.59
3.	1.416	11.774	15	1.2853	15	7.894	0.1487	2.19
					10	7.520	0.1108	2.76
			10	0.6707				

Es werden also unerwünschte Begleitstoffe zusammen mit wenig Enzym durch den Zusatz von ca. 70 Vol. % Aceton, bezogen auf die Ausgangslösung, beseitigt und der den Hauptteil an Amylase enthaltende Niederschlag wird durch neuerlichen Zusatz von Aceton in einer Menge von 50 Vol. % der Ausgangslösung gewonnen, wodurch man Präparate von Fz=ca. 2—4 erhalten kann. Nachdem die günstigen Mengenverhältnisse kennen gelernt waren, wurde der Auszug (2) in der Tabelle mit 70 Vol. % Aceton (z. B. auf 100 ccm 70 ccm Aceton) versetzt, wobei man durch Eiskühlung immer für niedrige Temperaturen sorgte. Die Fällung wurde in ca. 300 ccm fassender Zentrifuge so rasch als möglich in einigen Portionen abgeschleudert. Die Acetonlösung wurde jetzt weiterhin mit 50 Vol. % Aceton (bezogen auf das Volumen der Ausgangslösung) versetzt und der Hauptteil an Amylase rasch abzentrifugiert. Nach raschem Trocknen mit Aceton und Aether wurde das fast weisse Präparat über konzentrierter Schwefelsäure im Vakuum aufbewahrt. Je rascher man mit den einzelnen Operationen verfährt, desto hochwertiger werden die Präparate. Die Ausbeute betrug ca. 2g. Trockenpräparat von Fz=2.56 aus 200g. Weizenkleiematerial und Ausgangsauszug von Fz=0.3.

Mit diesen Trockenpräparaten setzte ich nun die Adsorptions-und Elutionsversuche fort, wie oben schon geschildert wurde. Aus mehreren durchgeführten Versuchen, wobei Fz=ca. 9—11 und 15—17 erreicht wurde, möge wieder hier ein Beispiel mitgeteilt sein.

0.1g. Enzympräparat wurde nach Verreiben mit Glasmehl mit insgesamt 50 ccm Wasser in Lösung gebracht. Diese Lösung versetzte man mit 15 ccm Tonerdesuspension, liess unter Rühren 1/4 Stunde die Adsorption vor sich gehen, zentrifugierte das Adsorbat ab und eluierte nach einmaligem Waschen 1/2 Stund lang mit 10 ccm Phosphatlösung + 40 ccm Wasser unter Eiskühlung. 50 ccm der Elution wurden mit 10 ccm Tonerdesuspension, die mit 4.5 ccm $\frac{n}{10}$ HCl angesäuert wurde, zum zweitenmal adsorbiert und das gewaschene Adsorbat mit 10 ccm Phosphatlösung + 40 ccm Wasser wieder eluiert. Auf diesem wege wurde portionsweise die zur Trockensubstanzbestimmung genügende Menge Elution gewonnen.

Es folgen die Belege:

Enzymmaterial	angew. ccm	g. Maltose nach 30′.	K	g. Tr. Sub. in 100 ccm	Fz
Enzymlösung 0.2%	5	0.6880	0.01136	0.2	2.56
Filtrat vom 1. Adsorbat	5	—			
1. Elution	5	0.5597	0.00954	0.04106	10.75
2. Elution	5	0.4813	0.00802	0.02068	16.36

Es ist noch zu erwähnen, dass bei der fraktionierten Acetonfällung ziemlich bedeutende Mengen Enzym verloren gehen dadurch, dass viel vom Präparat in den Zentrifugiergläsern an den Wandungen hängen bleibt, das schwer herauszubekommen ist, insbesondere wenn die Arbeit nicht rasch durchgeführt wird, wodurch unvermeidliche grössere Aktivitätsverluste eintreten, besonders im Vergleich mit der Adsorptionsmethode.

Beispiel :

Material:	g. Tr. Sub.	g. Maltose nach 30′.	Fz
20 ccm Auszug	0.9360	6.587	0.3
Acetonfällung	0.0543	3.856	2.58
zum Vergleich, Einmalige Adsorp und Elution	0.0687	5.698	3.22

c) Versuche mit Bleiacetatfällung.

Die Amylase wird durch Bleiacetat aus dem Auszug mit Eiweissstoffen zusammen vollständig ausgefällt und aus dem Niederschlag mit schwach alkalischen Lösungen wieder eluiert.

Aus mehreren Versuchen sei ein Beispiel gegeben: 20 ccm Auszug wurden mit 4 ccm gesättigter klarer neutraler Bleiacetatlösung gemischt und zentrifugiert. Der Niederschlag wurde mit Wasser einmal gewaschen und mit schwach alkalischen Lösungen in Eiskühlung eluiert.

Auszug	Elutionslösung.	angew. ccm	g. Maltose nach 30′.	g. Tr. Sub. in 100 ccm.	Fz.
20 ccm		5	0.639	1.694	0.297
Filtrat.		5	—		
	0.5 ccm $\frac{n}{5}$ NH_3 + 19.5 ccm Wasser	5	0.255		
	1.0 ccm 〃 + 19.0 ccm 〃	5	0.296	0.0584	3.62
	1.5 ccm 〃 + 18.5 ccm 〃	5	0.215		
	1.0 ccm $\frac{n}{10}$ NaOH + 19.0 ccm 〃	5	0.311		
	1.5 ccm 〃 + 18.5 ccm 〃	5	0.377	0.0872	3.16
	2.0 ccm 〃 + 18.0 ccm 〃	5	0.281		
	1 ccm Phosph. + 19 ccm 〃	5	0.361		
	2 ccm 〃 + 18 ccm 〃	5	0.475	0.1166	3.05
	3 ccm 〃 + 17 ccm 〃	5	0.517	0.1358	2.88

Man kann also durch Bleiacetatfällung und darauf folgende Elution ziemlich den gleichen Fz=Wert wie bei der einmaligen Tonerdeadsorption, erreichen, aber besonders bei der Elution mit NH_3 oder NaOH tritt ziemlich grosser Verlust der Enzymmenge ein. Die Elution mit NH_3 oder NaOH hängt wesentlich vom Zustand der Flockenbildung mit Bleiacetat ab, denn je feiner die Flocken sind, desto besser waren die Elutionen.

Durch die Phosphatlösung kann man eine grössere Enzymmenge eluieren, jedoch treten dabei immer schwach kolloide Trübungen auf, besonders wenn man eine grössere Menge Phosphat verwendet.

d) Adsorption mit Tonerde und Bleiacetat.

Durch alleinige Aneinanderreihung der Tonerde und Phosphatlösung kann man nicht über gewisse Wirksamkeiten hinausgelangen und zwar ist die Grenze schon bei dreimaliger Elution erreicht. Sie müssen deshalb mit anderen Adsorptions-oder Elutionsmitteln kombiniert werden. Bleiacetat bildet in der Phosphatlösung kolloides Bleiphosphat, wodurch es möglich ist, noch Begleitstoffe zu beseitigen. Kolloides Bleiphosphat adsorbiert auch auf saurer Seite und dabei wird die Amylase auch adsorbiert. Deshalb müssen die Mengenverhältnisse genau bestimmt werden. Zu den folgenden Versuchen wurde basische Bleiacetatlösung genommen, und zwar eine gesättigte klare Lösung.

Beispiel:

In 20 ccm 1. Elution zugegebene Bleiacetatmenge	angew. ccm	g. Maltose nach 30′	PH
	5 ccm Elution	0.7093	8.0
1.4	5	0.6074	6.4
1.6	5	0.5120	6.2

Aus zahlreichen Versuchen wird ein günstiges Mengenverhältnis als Beispiel hier gezeigt:

30 ccm Auszug wurden mit 30 ccm Tonerdesuspension versetzt, nach 1/4 Stunde zentrifugiert, das Adsorbat einmal gewaschen, dann mit 18 ccm Phosphatgemisch + 12 ccm Wasser 1/2 Stunde lang unter Eiskühlung und Turbinierung eluiert. 30 ccm der Elution wurden mit 2 ccm gesättigter klarer basischer Bleiacetatlösung versetzt und gut umgerührt und nach 1/4 Stunde zentrifugiert. Das Filtrat wurde mit 6 ccm Tonerdesuspension, welcher 1.2 ccm $\frac{n}{10}$ HCl zugegeben wurde, zum zweitenmal adsorbiert und nach der gleichen Behandlung mit 6 ccm Phosphatgemisch + 24 ccm Wasser in gleicher Weise eluiert. 30 ccm der zweiten Elution wurden mit 0.9 ccm Bleiacetatlösung versetzt und weiter in gleicher Weise behandelt. Das Filtrat wurde mit 3 ccm Tonerdesuspension unter Zusatz von 1.2 ccm $\frac{n}{10}$ HCl zum drittenmal adsorbiert und nach der gleichen Behandlung mit 3 ccm Phosphatgemisch + 27 ccm Wasser wiederum eluiert. Je mehr die Reinigung zunimmt, desto schärfer muss man die Elution zentrifugieren, um ganz klare Elution zu gewinnen.

Es folgen die Zahlenbelege:

Material.	angew. ccm	g. Maltose nach 30′	K	g. Tr. Sub. in 100 ccm Enzymlösg.	Fz.
Auszug	5	0.836	0.01546	3.640	0.1911
1. Elution	5	0.710	0.01264	0.1275	4.46
2. Elution	5	0.4912	0.008189	0.02596	14.25
3. Elution	5	0.372	0.006025	0.01120	24.16

Der Erfolg der einmaligen Elution und darauf folgender zwei aneinandergereihter Adsorptionen und Elutionen mit Tonerde, Bleiacetat und Phosphatgemisch war nun folgender:

Die zweite Adsorption und Elution führte von den Auszügen mit einem Fz=0.2–0.4 zu einem Fz=ca. 13–15. Die dritte Adsorption und Elution erhöhte das Verzuckerungsvermögen auf Fz=ca. 23—26, was unter diesen-durchgeführten sämtlichen Versuchen der höchste Wert war.

Was bereits bei den Vorversuchen wichtig war, musste in gleicher Weise auch bei den zweiten und dritten Adsorptionen und Elutionen neuerlich geprüft werden. Insbesondere waren jeweils die günstigen Mengenverhältnisse von Adsorbens und Sorbendum neu zu ermitteln.

V. Analyse des Reinpräparates.

Nachdem man zu einem nicht leicht zu übertreffenden Höchstwert gelangt war, musste man, um die gereinigten Präparate zu analysieren, grosse Mengen Auszug auf die gleiche Art portionsweise reinigen und im Vakuum eindampfen. Man erhielt so insgesamt eine Menge Trockensubstanz von 1.1906 g. Sie wurde mit heissem Wasser aus der Platinschale quantitativ herausgenommen und auf 50 ccm aufgefüllt. Von der gut durchgeschüttelten Lösung wurden mit der Pipette gemessen Teile entnommen und folgende Bestimmungen durchgeführt.

Um die phosphatfreie Trockensubstanz zu bestimmen, wurde ein Teil davon zur Phosphorbestimmung gebracht. Es enthielt 1 ccm der Lösung 0.002693g. phosphatfreie Trockensubstanz.

Um den Kohlehydratgehalt zu bestimmen, wurden 20 ccm davon mit 4 ccm HCl von sp. Gew. 1.125 und ca. 20 ccm Wasser 3 Stunden lang im kochenden Wasserbad unter Rückfluss erhitzt. Nach dem Abkühlen wurde neutralisiert, auf 100 ccm aufgefüllt und die Zuckerbestimmungen ausgeführt. Der Kohlehydratgehalt wurde aus dem Dextrosewert nach Inversion und Multiplikation mit 0.9 berechnet.

Der Stickstoff wurde nach der Mikrokjeldahlmethode[31] bestimmt.

Das Ergebnis war folgendes:

Proteingehalt:	im Präparat von Fz = 2.5	35.13%
	im Präparat von Fz =25.5	69.89%
Kohlehydratgehalt:	im Präparat von Fz = 2.5	16.82%
	im Praparat von Fz =25.5	26.94%

Färbungsreaktionen des Präparates: Xanthoprotein R., Millonsche R., Biuret R., Liebermann'sche R. und Adam-Kiewiczsche R. sind alle sehr deutlich eingetreten.

Durch den Reinigungsvorgang fand also eine erhebliche Anreicherung der Stickstoffsubstanzen und eine dementsprechende Verarmung an Kohlehydraten statt.

Zum Vergleich bringe ich die Analysenwerte, welche Lüers und Sellner an ihren reinsten Malzamylasepräparaten fanden:

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31) F. Pregl: Die quantitative organische Mikroanalyse. 113.

	Nishimura Asperg. oryzae	Lüers u. Sellner Malzamylase
Proteingehalt	69.89%	74.4%
Kohlehydratgehalt	26.94%	28.07%

Die Werte liegen ziemlich nahe beieinander.

Dieses energische Zusammenhalten des Komplexes Eiweiss–Enzym ist bemerkenswert. Es kann aber zur Zeit nicht gesagt werden, dass die Amylase selbst ein Eiweisskörper sei, eine endgültige Behauptung nach dieser Richtung wird erst zu fällen sein, wenn weitere Reinigungsversuche, die zu bedeutend aktiveren Präparaten führen müssen, vorliegen werden. Dass aber das Enzym auch in Aspergillus oryzae in naher Beziehung zu Eiweisskörpern steht, beweisen diese Versuche ebenso wie jene an der Malzamylase von Lüers und Sellner.

Zusammenfassung :

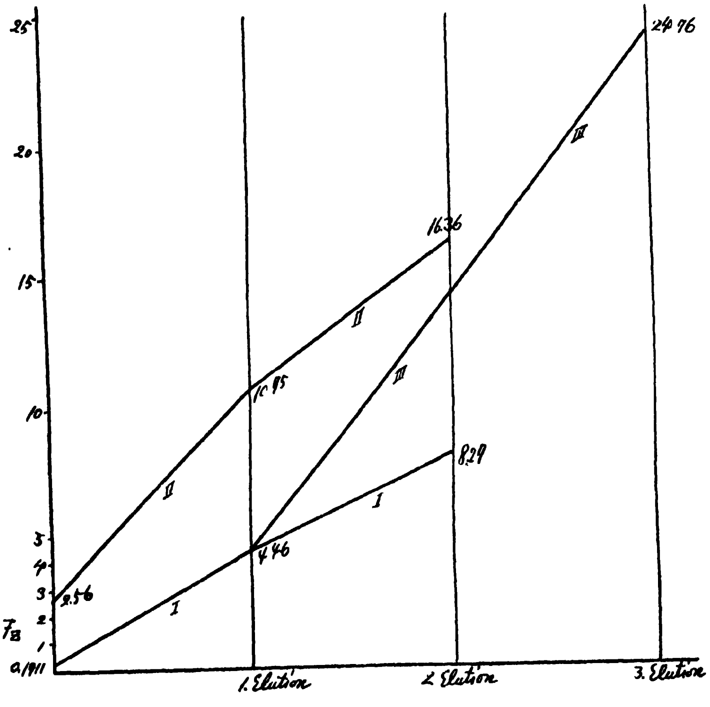
Die von R. Willstätter und seinen Mitarbeitern ausgearbeiteten und von H. Lüers und E. Sellner zur Reinigung der Malzamylase verwendeten Methoden der Adsorption und Elution besonders mit Tonerdesuspensionen und Phosphatgemischen sind auch zur Reinigung der Amylase aus Aspergillus oryzae gut brauchbar. Das kolloide Aluminiumhydroxyd nimmt aus schwach saurer Lösung das Enzym vollständig auf. Aus diesem Adsorptionsverhalten geht der amphotere Charakter des Komplexes, den die Amylase mit Begleitstoffen von Eiweissnatur bildet, hervor. Aus dem Tonerdeadsorbat lässt sich das Enzym in reinerem Zustand mit sehr schwach alkalischer Phosphatlösung von $P_H = 8.0$ eluieren. Durch Aneinanderreihung von zwei Tonerdeadsorptionen und zwei Elutionen gelingt es, vom Auszug von Fz = ca. 0.2—0.4 ausgahend, den Reinheitsgrad des Enzyms auf Fz = ca. 3—5 bezw. 7—9 zu steigern. Eine dritte Adsorption und Elution gelang in keinem Fall. Eine weitere Steigerung des Reinheitsgrades lässt sich dagegen erzielen, wenn man von trockenen durch Acetonfällung erhaltenen Präparaten mit bereits höherem Wirkungswert ausgeht. Durch zwei Reinigungen gelang es, vom Trockenpräparat von Fz = ca. 2—4 ausgehend, die Wirksamkeit auf Fz = ca. 9—11 bezw. 15—17 zu steigern. Die Methode hat aber den Nachteil, dass sie ziemlich grosse Verluste des Enzyms bedingt und trotz weiterer Vorfolgung keinen höheren Wert mehr erreichen lässt.

Auch Aneinanderreihung von zwei Tonerdeadsorptionen und zwei Elutionen führt nicht wesentlich weiter. Durch Kombination von Tonerde und Bleiphosphatadsorptionen und Phosphatelutionen gelang die bisher höchste Wirksamkeitssteigerung und zwar, vom Auszug von Fz = ca. 0.2—0.4 ausgehend, durch zweimalige Reinigung auf Fz = ca. 13—15 und weiter durch eine dritte Reinigung auf Fz = ca. 23—26. Wenn man von noch hochwertigeren Auszügen ausgehen könnte, könnte man sicher noch höhere Wirksamkeitssteigerung erreichen, obwohl Anfangskonzentration und Wirksamkeitssteigerung nicht parallel gehen.

Die Wirksamkeitssteigerungen werden hier nochmals anschaulich zusammengestellt.

Reinigungsmittel	Ausgangsmaterial	Fz	1. Elut. Fz	2. Elut. Fz	3. Elut. Fz
I. Tonerde und Phosphat	Auszug	0.1911	4.46	8.27	
II. Tonerde und Phosphat	Tr. Praparat	2.56	10.75	16.36	
III. Tonerde, Bleiacetat u. Phosphat	Auszug	0.1911	4.46	14.25	24.16

Sie werden graphisch folgend gezeigt:



Die Analyse der reinsten Präparate ergab einen Proteingehalt von 69.89 % und einen Kohlehydratgehalt von 26.94% der phosphorsalzfreinen Substanz. Gegenüber dem Ausgangsamaterial hatte der Stickstoffgehalt stark zu–, der Kohlehydratgehalt stark abgenommen. Das Ergebnis stimmt gut mit den Analysenwerten von Lüers und Sellner an der Malzamylase überein.

Die reinsten Präparate zeigen deutlich alle Färbungsreaktionen des Eiweisses.

Ein bestimmter Eiweisskörper scheint also zum Enzym in näherer Beziehung zu stehen. Wie schon oben erwähnt, soll damit keineswegs behauptet

werden, dass die Amylase selbst ein Protein sei. Weitere endgültige Versuche müssen hier erst Klarheit bringen.

Um meinen besten wert von Fz=26 mit dem von Takamine dargestellten Präparate, das 43 (bei 21°C.) Lintner-Einheiten hatte, vergleichen zu können, und ihn gleichzeitig in einem bekannten Masse verständlich zu machen, rechne ich ihn auf die von Lintner eingeführte Wertung um. Nach Lintner wird die diastatische Kraft ausgedrückt durch die Menge Maltose, welche von 100 g. Enzym-Trockensubstanz in 30 Minuten bei einer Temperatur von 20°C. gebildet wird.

0.000568g. meines Präparates lieferte in 30 Minuten bei 20°C. 0.4025g. Maltose. 100g. des Präparates erzeugen folglich rund 71000g. Maltose, oder das Präparat hat eine diastatische Kraft von 71000 Lintner-Einheiten.

STUDIES ON PROTEINS VI.

THE INFLUENCE OF SALT ON THE POINT OF OPTIMUM FLOCCULATION OF RICE-GLUTELIN.

(Contribution No. 7 from the Laboratory of Nutritional Chemistry, Dept. of Agriculture, Kyoto Imperial University)

By

Kinsuke KONDO, Tunetomo HAYASHI and Takashi MATSUSHITA.

(Received Oct., 1st., 1926.)

A. The point of optimum flocculation of the rice-glutelin is dependent upon the salt in the solution. We attributed this cause to the difference of the protein-ionizing forces of ions derived from the salt. We examined as to whether our theory holds good for $NaCH_3COO$, $NaCl$, KCl and $LiCl$ or not. The proteins, used in the present work, are rice-glutelin No. 1 and No. 2.

B. The 5c.c. of the concentrated sodium rice-glutelinate solution is mixed with an acetate acetic acid solution in such a manner as to insure that the concentration of sodium acetate be constant and that of acetic acid be variable in the solution. The rice-glutelin flocculates out in various quantity. These results show us that in the range of 0.05—0.3n the higher the concentration of sodium acetate in the solution become, the farther the point of optimum flocculation or apparent iso-electric point of this rice-glutelin goes to the acidic side, and it is variable in proportion to the salt concentration. This is not identical with the results of Michaelis,(1) and the optimum flocculation does not mean the complete flocculation.

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(1) Michaelis, Biochem. Zt. 103, 178 (1920).

But the maximum amount of the flocculated rice-glutelin is not changeable in all. the experiments, in the range of experimental error.

Therefore we may conclude that the amount of maximum precipitation of this protein is independent of the concentration of the sodium acetate.

But the amount of the precipitated protein is less than the entire amount in the solution. In other words the optimum flocculation does not mean the complete flocculation and a part of this protein is dissolved in the solution after optimum flocculation.

We examined also the behavior of the rice-glutelin in the solution containing another salt mixed with sodium acetate and cast one glance the influences of NaCl, KCl and LiCl on the flocculation of this protein.

According to the results we see that up to the point when the concentration of the used salt increases to 0.5n, the higher the concentration of the salts becomes, the less the amounts of precipitated rice-glutelin.

But if the salt concentration increases to more than 0.5n, the amount of flocculated protein again increases. And in the 0.1n sodium acetate solution containing 0.01n of the chloride the rice-glutelin can flocculate at its greatest and more than in the acetate solution, free from the other salt. But even in such a solution the rice-glutelin can not flocculate completely.

C. We have elucidated these phenomena by showing the difference among the protein-ionizing forces of ions derived from the salts. These forces are peculiar to the kind of salt and its concentration and the nature of the protein. Therefore the apparent iso-electric reaction of a protein difficult of solution is variable according to the salt used and its concentration.

STUDIES ON PROTEINS VII.

ON THE REFRACTIVE INDICES OF PROTEIN SOLUTION PART I.

(Contribution No. 8 from the Laboratory of Nutritional Chemistry, Dept. of Agriculture, Kyoto Imperial University).

By Kinsuke KONDO and Tunetomo HAYASHI.

(Received Oct. 1st., 1926.)

A. Robertson(1) determined the refractive indices of the protein solution with the aim of determining more accurately the amount of protein in the solution. Thereupon he proved that refractive indices of the protein solution run accurately with the concentration of protein according to the following formula:

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(1) T. B. Robertson:- Jour. Phys. Chem., 13, 469 (1909).
The other results are summarized in his book; Phys. Chem. of Proteins 1918.

$$n - n_1 = a \times c$$

"n" and "n_1" represent the refractive indices of the solution and solute respectively; "c" is the protein concentration in the solution (number of gms. of protein in 100 cc.) and "a" is the special constant for the protein.

After Robertson, the change of refractive indices of the aqueous solution after dissolving of the protein is a function of the space, which is occupied by the protein molecule. And the molecular volume is the additive function of the atomic volume. Hence the molecular refraction is the additive function of the atomic refraction. The protein molecule is an associated substance of a number of atoms, and the volume of protein is almost inchangeable altho a few atoms of protein are substituted for other atoms or any atoms or any atom or atom group may be added to the protein molecule. Therefore the change of the refractive indices of the aqueous solution by the dissolution of the protein is independent of the nature of acid or alkali which may combine with protein or their combining proportion, because the molecular refraction is the additive function of the atomic refraction.

Our present knowledge in this field however teaches us that the atomic refraction is dependent upon the valency, the position in the molecule and the iogenic condition of the atom. Possessing this knowledge we may easily suppose that the molecular refraction is changeable according to the chemical and physical condition of the molecule and ints constituents, i. e., the atoms, because, as we say, the molecular refraction is the additive function of the atomic refraction.

That is, the refractive indices of the solution are the function of the molecular volume of the solute as well as that of its construction and its condition. Moreover, we may consider that the protein is accompanied by an intermolecular change in its molecule on the opposite sides of its iso-electric point.(2) Notwithstanding this change, will the refractive indices of the aqueous protein solution be independent of the nature of acids or alkali which combine with protein and their combining proportion? Robertson also adds that his theory can only hold good for the special solvent, i. e., the diluted aqueous solution of acids or alkali. Thus, for example, his theory can not hold good for an alcoholic protein solution.

Now the refractive indices of the solution are not only dependent upon the nature and wave length of the physical condition of the solvent and solute, accompanied by the change of the latter's concentration, but the problem will become more complicated and complex if we take into consideration the consumptive absorption of light and conservative smoothering of the solution. It is, therefore, not to be wondered at that the great studies in this field could not be governed throuout by theory, even tho the work was concerned with a real solution or a colloidal solution.

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(2) J. Loeb:- Protein and the Theory of Colloidal Behavior, p. 33 (1922)

B. a. On Rice-glutelin.

Rice-glutelin No. 2 is used for the present work. The sodium rice-glutelinate solution is prepared in such a manner as that the concentration of sodium hydroxide become 0.02n and the amount of the protein nitrogen is 4.0199g in 1L. of the solution.

With this solution and its diluted solution the refractive angle is under a Na-light, observed, by means of Pulfrich's refractometer which keeps the observed solution at 18°±0.1C. with a current of hot water in its pipe. The results are tabulated as follows.

Table 1.

Sodium Rice-glutelinate

a g.of Rice-glutelin-N in 1 L. of Solution	b g. Equivalent of Rice-glutelin-N	n Refractivity	v	μ $\frac{n-n_w}{b}\times 10^3$	α $\frac{\mu}{\mu_\infty}$	paH
4.0199	0.2869	1.33732	3.4855	15·999	83.33	11.010
2.0100	0.1434	1.33501	6.9735	15.900	82.81	11.098
1.0050	0.0717	1.33391	13.9470	16.457	85.71	10.910
0.5025	0.0358	1.33333	27.9320	16.750	87.29	10.662
0.2513	0.0179	1.33304	55.8640	17.318	90.20	10.462

· In the table "a" and "b" represent the number of grams and g-equivalent of rice-glutelin-nitrogen in 1L. of the solution respectively. "n" and "n_w" are the refractive indices of the solution and water.

"V" represents the number of liters which contain a g-equivalent of the protein nitrogen. $\mu=\frac{n-n_w}{b}$ is the refractivity of the solution which contains one g-equivalent of rice-glutelin in 1L. of the solution and we call this the nitrogenequivalent refractivity of the protein.

If we take the nitrogen-equivalent refactivity as ordinate and $\frac{1}{V}$ as abscissa, we can trace the μ -V curve as in the following figure.

"α" represents the value of $\frac{\mu}{\mu_\infty}$, in which μ_∞ is estimated after the figure and is called the nitrogen-equivalent refractivity at an indefinite dilution.

Observing the above table, it comes to our mind that the action of the rice-glutelinate as well as that of the alkali-caseinate resemble a typical neu-

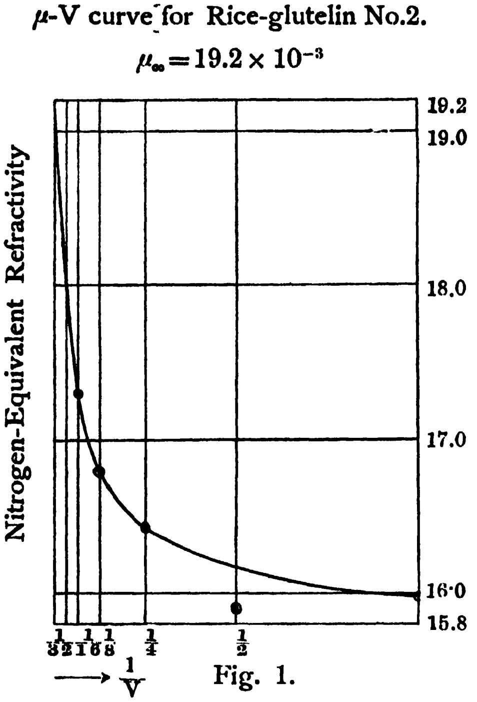

Fig. 1.

tral salt because the value of paH rises slowly with increasing dilution.

Therefore we might better consider that α represents the degree of dissociation of rice-glutelinate than that it shows only the change of the refractivity of the rice-glutelinate solution with its dilution.

B. b. On Casein.

We can see the same phenomena with casein as well as with rice-glutelin. The casein (Kahlbaum) is treated as we treated the riceglutelin and the sodium caseinate solution contains 7.2682g of casein-nitrogen in 1L. of the solution. The refractivity of this solution are observed under a Na-light at 18°C. The results are summarized in Table 2.

Table 2

Sodium caseinate

a g. of Casein-N in 1L. of Solution	b g. equivalent of Casein-nitrogen	n Refractivity	V	μ $\frac{n-n_w}{b} \times 10^3$	α $\frac{\mu}{\mu_\infty}$	paH
7.2682	0.5188	1.34167	1.9276	17.232	81.75	6.378
3.6341	0.2594	1.33721	3.8550	17.271	81.93	6.480
1.8170	0.1297	1.33499	7.7101	17.425	82.66	6.688
0.9085	0.0618	1.33389	15.4202	17.901	84.92	6.766
0.4543	0.0324	1.33334	30.8404	18.827	89.31	7.024

All signs in Table 2 means the same as in Table 1. The μ-V curve for the sodium caseinate is figured in the same way as in the figure. By means of this figuration the value of μ_∞ is extrapolated and then α is calculated as in the table.

C. Our results with either rice-glutelin or casein are not in accordance with Robertson's formula, etc.(3) That is, the refractive indices of the protein

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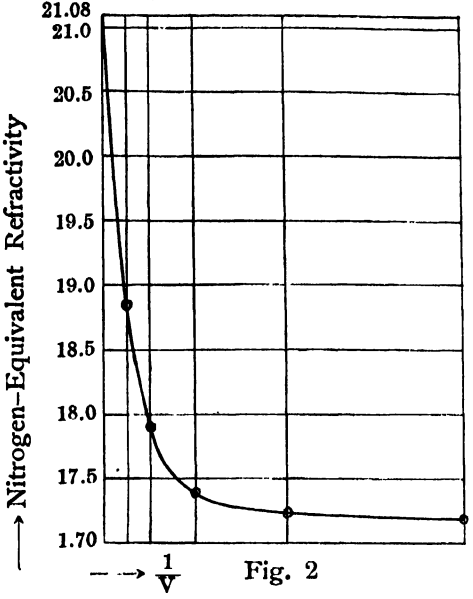
(3) Walpole, G. S:- Koll. Zt., 13, 211 (1913) Wington, R:- Koll. Beih., 7, 251 (1915) Wirgner, G:- Koll. Zt., 20, 7 (1917)



μ-V curve for Casein

$\mu_\infty = 21.08 \times 10^{-3}$

Fig. 2

aqueous solution is not accurately proportional to the concentration of protein. After Robertson's theory, the protein molecule has a certain specific refraction in an aqueous solution, which is independent of the protein concentration. (The diluted acid or alkali solution)

We, however, believe that the protein salt dissociates in an aqueous solution, and the degree of the dissociation is dependent upon the protein concentration as in the case of typical salt, and further more the ionized molecule has a different refractive power from the non-ionized one.(4) Accordingly the refractivity of the protein in aqueous solution is changeable according to the protein concentration.

If this supposition holds good for our case, it is, of course, natural that our experimental results could not be governed by Robertson's formula.

Under the above supposition, we can elucidate the change of refractivity of the protein aqueous solution by the ion theory of the protein molecule and can confirm with certainty that an ionized protein has not the same refractive power as that of a non-ionized one. Therefore, the determination of the Nitrogen Equivalent-Refractivity of the protein is able to show us the degree of dissociation of the protein salt.

After this principle we can say conclusively that sodium rice-glutelinate can dissociate highly as well as sodium caseinate more than about 80 per cent.

If we expand our theory on the refractivity of the protein solution, it will result in the conclusion that to an ampholyte such as protein belong different nitrogen-equivalent refractivities according as the protein behavior is that of a cation or an anion.

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(4) Le Blanc:- Zt. Physik. Chem. 4, 553 (1889) Ibid, 19, 261 (1896)

STUDIES ON THE DIGESTION OF FOODS. (PART I)

ON THE ETHER EXTRACT OF EXCRETORY FROM DIGESTIVE ORGANS.

By Shigeo SUZUKI and Tetsuo MUROTA.

We have attempted to find out the amounts of nitrogen compounds, ether extracts, and crude ash, which, on determining a digestive coefficient of foods and feeding stuffs by animal experiment, comes to mix in the feces from the digestive organs, and to creat a new basis for the calculation of a true digestion coefficient, which can replace the old, mere external coefficient. So two dogs were fed with the fat free rice powder, horse meat powder and common salts, added with some fiber, for we thought that the excretory products of digestive organs must vary according to the digestion coefficient of every food. To this purpose the filter paper was destroyed to fiber by water.

Each period, our experiment was done, consists of ten days, and we determined the difference of the ether extract in feces, which is considered to come from the digestive organs by each different coefficient.

The following figures are its results.

Fiber in the food.	Digestion coefficient of dry matter.	Total ether exract in the feces.	Ether extract in the fece-dry matter.
	%	gr.	%
A—dog, non fiber	97.241	3.851	7.109
B— , 〃 〃	97.281	3.764	7.051
A. B—, 〃 〃 (av.)	97.261	3.808	7.080
B— , 0.5% 〃	96.614	4.654	6.966
A— , 1〃 〃	95.879	4.259	5.265
B— , 1〃 〃	96.053	4.191	5.354
A, B—, 1〃 〃 (av.)	95.966	4.225	5.310
B— , 2〃 〃	94.981	3.974	3.953
A— , 3〃 〃	93.841	3.927	3.152
B— , 3〃 〃	93.827	3.921	3.141
A, B—, 3〃 〃 (av.)	93.834	3.924	3.147
B— , 4〃 〃	92.907	3.885	2.682
A— , 5〃 〃	91.700	3.817	2.230
A— , 7〃 〃	89.865	3.260	1.531
A— , 10〃 〃	87.383	2.349	0.861

(1) The amount of the ether extract which mixes in the feces is different with the variation of the digestion coefficient. Generally the less the fiber in food is, the more the amount of excretory products and the

percentage of the ether extract in the dry matter of the feces is.

(2) The percentage of the ether extracts in the dry matter of feces decreases with the decline of the digestion, and its curve are regular. Therefore, when we know a digestion coefficient of total dry matter which was eaten, it is easy to see the quantity of the ether extract comes from the digestive organs, and from this we can calculate a true digestion coefficient of fat.

(3) On the digestion the effect of the increasing amount of fiber in food is not large, but in case of the crude protein and crude ash, it is larger than in case of the starch. And yet this is not always reliable, for it is a mere external digestion coefficient, in which the mixture from the organ is neglected.

Concerning the mixture of the crude protein and crude ash, we are now in the course of research.

ON THE FERMENTATION PRODUCTS OF GLUCOSE BY *Oidium Lupuli.*

By Yusuke SUMIKI.

(Agricultural chemical laboratory, Tokyo Imperial University).

Oidium Lupuli Mathews et Lott is a fungus which occurs in wet hops or in "Hatchomiso-koji".

Up to the present, several investigations on Oidium Lupuli have been reported by Lindner (Woch. f. Brauerei, II, 1312, 1894), G. Kita (Deut. Ess. Indust., Dec., 1913), T. Akagi, I. Nakajima, K. Tsugane (J. Coll. Agr. Imp. Uni. Tokyo, 5, 263, 1915) and Y. Nishiwaki (Jōzōzâshi, 434, 17, 1911; Centbl. f. Bakt., Abt. II, 63, 25, 1924).

I. Condition of culture.

Oidium lupuli was cultured at 30°C for 20—37 days in 39L of the following medium.

Glucose	10.00 g.
Peptone	0.10 g.
K_2HPO_4	0.015g.
KH_2PO_4	0.015g.
$MgSO_4$	0.010g.
$CaCl_2$	0.010g.

$FeCl_3$, NaCl	trace.
Dist. water	100ccm.

The amount of glucose consumed was estimated by Bertrand's method at several intervals.

Days after inoculation.	Glucose consumed in 100ccm. of medium.
16	4.75 g.
23	5.95 g.
30	6.98 g.
37	7.73 g.

II. Isolation and identification of fermentation products.

By steam distillation, volatile substances in the medium are isolated from non-volatile substances [residue (A), distillate (B)]. The distillate is added with the excess of barium carbonate and boiled. After cooling and filtering, the filtrate is distilled for the isolation of alcohol and aldehyde from barium salt of volatile acid [residue (C), distillate (D)].

(1) Ethyl alcohol.

A fraction, b. p. 78°C, is obtained from the distillate (B) by repeating fractional distillation. This fraction, on treatement with the theoretical quantity of phenyl isocyanate, yields phenyl urethane, white long needle, m. p. 52°C.

0.0853g. subst. gave 6.15cm³ N_2 (15.5°, 762.4mm.)

Calculated for $C_9H_{11}O_2N$	N 8.49%.
Found	N 8.56%.

(2) Acetaldehyde.

A fraction is obtained by repeating fractional distillation from the distillate (B) which gives the reaction of aldehyde with Schiff's reagent. This fraction also gives the following colour reactions (see author's report, Bull. Agr. Chem. Soc. Jap., 2, 24, 1926).

(a) Reaction of Windish.
(b) Reaction of Jeau.
(c) Reaction of Pittarelli.
(d) Reduction of ammoniacal silver nitrate solution.

By the addition of dimedon, this fraction acetaldimedon, m. p. 137—8°C. The m. p. of mixture with pure acetaldimedon shows no depression.

(3) Succinic acid.

The residue (A) is concentrated under diminished pressure, is acidulated with phosphoric acid and extracted with ether. After removing ether and recrystallising from hot water, succinic acid, m. p. 184°C, is obtained.

0.1080g. subst. gave 0.1602g. CO_2 and 0.0512g. H_2O

Calculated for $C_4H_6O_4$	C 40.67%	H 5.08%.
Found	C 40.45%	H 5.26%.

(4) Acetic acid.

The residue (C) is evaporated to dryness, added with phosphoric acid to set volatile acid free and distilled. The distillate, on treatment with yellow mercuric oxide, gives white mercuric acetate.

0.0620g. subst. gave 0.0152g. SHg.
Calculated for $C_4H_6O_4Hg$ Hg 62.96%.
Found Hg 62.84%.

III. Quantitative determination of fermentation products.

The four substances above described were identified as the fermentation products, but the latter three were produced in so small amounts that I determined quantitatively only ethyl alcohol by the ordinary distillation method.

The conditions of culture are all the same as mentioned above (see I) except the quantity of peptone, the source of nitrogen, which is indicated by the following table.

	Quantity of peptone in 100ccm of medium.	Glucose : Peptone.	Maximum yield.	Days after inoculation.	Wt. of fungus.
1.	0.1g.	100 : 1	3.00vol%	31	0.11g.
2.	0.5g.	100 : 5	4.29 "	19	0.26g.
3.	1.0g.	100 : 10	4.18 "	16	0.36g.
4.	3.0g.	100 : 30	1.82 "	14	0.57g.
5.	5.0g.	100 : 50	1.20 "	12	0.58g.

IV. Conclusion.

It has been found that ethyl alcohol is the greater part of fermentation products from glucose and also a small amount of succinic acid, slight quantities of acetic acid and acetaldehyde are produced along with ethyl alcohol. 3.40g. of ethyl alcohol is the maximum yield from 10g. of glucose when the ratio of glucose to peptone is about 20 to I.

ON THE DIFFERENCES OF THE BREWING BARLEY ACCORDING TO SPECIES.

I. THE INVESTIGATIONS OF THE PROTEINS.

By Yukihiko NAKAMURA.

(Received Dec. 10th., 1926.)

Introduction.

The use of barley in Japan is very wide, not only as food for man, and farm animals, but also for the brewing of beer. The amount of its consumption especially for the brewer's purpose, with the improvement of the fermentation industry, increases year after year. Consequently, barley is now ranking among the more important products in our agricultural world. From statistics, it can be seen that the production of barley in this country is about 8,800,000 Koku valued at 10,000,000 Yen per annum and from the standpoint of the technical industry, the amount of thc consumption of barley for brewing is the largest. The import of malt is disproportionately larger than the import of barley, indicating that there must be some differences in the barley and the malt produced at home and abroad. The very large amount of consumption of barley for brewing purposes tells us how a study of the barley might be useful for the brewing. Recentry some authors were able to find out the physico-chemical specificities of oryzcnins according to the varieties of the rice. The present investigation is aimed to find out, if any, marked specificities in the proteins of the barley.

Investigations upon barley which have hitherto been carried out and are already numerous, can be classified into 9 parts; (1) upon the method of the estimation, (2) upon the nutrition and the nutritive value, (3) upon the relations among the soil, the manure and the growth of the plant, (4) upon the relations betweén the growing period and the constituents of the plant, (5) upon the fermentation, (6) upon the starches, (7) upon the fats, (8) upon the proteins and (9) upon the enzymes.

But concerning the protein and the fermentation of the barley, almost all the investigations are limited to the determinations of the hydrolyses products of the proteins, or the results of feeding experiments with the proteins. The effects of the species of the barley upon the proteins or the differences of the proteins derived from the different species of the barley are not yet studied. If the problem could be made clear and the effects of the different species of the barley were known, it would tell us another way and direction of agricultural planning.

Conclusions.

The barley widely used among the brewer in this conutry are Golden Melon, Chevalier and Hokudai No. 1, so the present investigation was carried out with these 3 species of barley. The results of the present physico-chemical and chemical investigations of the proteins obtained from these 3 species of barley, using the yield of three successive years, are as follows:–

(1) Using 1,000 kernels each, no regular relationship could be found among the characters of the three species of barley, so far as they concern weight and specific gravity of the barley grains.

(2) On the chemical analyses of the powder of the grains, the total nitrogen and the protein nitrogen content are the least in Golden Melon, and the most in Chevalier with Hokudai No. 1 lying between the two.

(3) The isolation and determination of four kinds of nitrogen of these three species always shows the smallest amount of nitrogen soluble in 10 % NaCl solution and the amount increases according to the order of the water soluble, the 70 % alcohol solution soluble and the 0.2 % NaOH solution soluble nitrogen.

The quantities of 10 % NaCl solution soluble nitrogen decrease in the order Golden Melon, Hokudai No. 1 and Chevalier. The 0,2 % NaOH solution soluble nitrogen decreases in the order Hokudai No. 1, Golden Melon and Chevalier.

(4) The ash content of the proteins increases in the order, hordein, 10 % NaCl solution soluble protein and glutenin. As for the species of the barley, Golden Melon has the minimum ash content, Chevalier medium, and Hokudai No. 1 the maximum. This appears to be a constant relation found among the species of the barley and among the kinds of the proteins.

(5) Among the species of the barley, there is a tendency that the content of carbon and hydrogen of the protein always follow and that of oxygen is always contrary. The content of the nitrogen of the protein is markedly different among the species of the barley. That of the 10% NaCl solution soluble protein and the glutenin is the maximum in Hokudai No. 1, medium in Chevalier and the minimum in Golden Melon while that of the hordein is maximum in Golden Melon, medium in Chevalier and minimum in Hokudai No. 1. The content of the nitrogen of the hordein is the smallest, the glutenin medium and the 10 % NaCl solution soluble protein the maximum.

The sulphur content of the proteins is not regular, but it can be seen that of the hordein is the highest and the glutenin the lowest.

(6) The amount of free amino nitrogen of the 10 % NaCl solution soluble protein is the greatest in Golden, medium in Hokudai No. 1 and the smallest in Chevalier, and that of the glutenin, the greatest in Chevalier, medium in Golden Melon and the smallest in Hokudai No. 1. The amount of free amino nitrogen of the hordein is always greater in Chevalier than in Hokudai No, 1, and in Golden Melon it is irregular. The hordein contains less free amino nitrogen than the 10 % NaCl solution soluble protein and the glutenin, while the 10 % NaCl solution soluble protein seem to contain more than the glutenin.

(7) Differences are observed among the content of the hydrolytic products of the 10 % NaCl solution soluble protein, of the hordein and of

the glutenin according to the species of the barley.

(8) The changes of the turbidity and the surface tension of the protein in an alkaline solution when titrated with HCl are observed. In the case of the water soluble protein, the maximum quantity of HCl in order to attain greatest surface tension and turbidity is required by Golden Melon, and in the case of the 10 % NaCl solution soluble protein, the required quantities of HCl are decreased in the order Hokudai No. 1, Chevalier and Golden Melon. In the case of the hordein, that of Golden Melon requires the maximum amount of HCl, next Hokudai No. 1 and Chevalier requires the minimum amount. Using glutenin, the quantities of HCl required decrease in order Hokudai No. 1, Golden Melon and Chevalier.

(9) From the results of the preceding experiments, i. e. the changes of the turbidity and the surface tension and the results of the determination of free amino nitrogen, it can be deduced that the P_H value of the isoelectric point of the water soluble protein is the lowest in Golden Melon, that of the 10% NaCl solution soluble protein decreases in the order Golden Melon, Chevalier and Hokudai No. 1, that of the hordein decreases Chevalier, Hokudai No. 1 and Golden Melon, while that of the glutenin decreases Chevalier, Golden Melon and Hokudai No. 1.

(10) The specific rotatory power of the water soluble protein in an alkaline solution is the largest in Golden Melon, that of the 10 % NaCl solution soluble protein alkali solution decreases in the order Golden Melon, Chevalier and Hokudai No. 1, and that of the hordein in an alkaline solution decreases in the order Chevalier, Hokudai No. 1 and Golden Melon.

QUINOLINE AND ACRIDINE SYNTHESIS.

By Konomu MATSUMURA.

(Received Oct 2nd., 1926.)

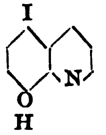
I. 7-Iodo-5-amido-8-hydroxy-quinoline.

7-Iodo-5-nitro-8-hydroxy-quinoline (I) was obtained by iodination of 5-nitro-8-hydroxy-quinoline. Orange needles. F. 249° (decomp.)

7-Iodo-5-amido-8-hydroxy-quinoline was obtained by reducing (I) with $SnCl_2$ and conc. HCl. Light yellowish needles. It softens at ca. 147° and melts at 157°. Picrate: reddich brown needles, F. 159° (decomp.).

II. 5–Iodo–8–hydroxy–quinoline.

A dilute aqueous solution of the sodium salt of 8–hydroxy–quinoline was treated with an aqueous solution of a molecular quantity of indine and the same quantity of KI at room temperature. The product was found to be a mixture of 5–Iodo– 8–hydroxy–quinoline (I) and 5–7–di–iodo–8–hydroxy–quinoline (II).



(I) was separated from (II) by its easier solubility in ethyl alcohol than the latter.

(I) was crystallised from alcohol into colourless prisms. F. 127—8°. Methiodide: brown needles with metallic luster. F. 142°. Readily soluble in hot alcohol, scarcely in hot water, and insoluble in ether.

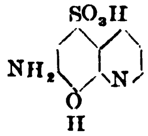
III. 7–Amido–8–hydroxy–quinoline and its related compounds.

8–Hydroxy–quinoline–5–sulfonic acid (I) was prepared by the Claus's method (J. pr. [2] 41, 33). F. 322—3°.

7–Benzen–azo–8–hydroxy–quinoline–5–sulfonic acid (II) was obtained by coupling (I) with diazo–benzene–chloride.

Long needles of dark red colour, darkening at 268° and melting at 310°· Mono Na–Salt: orange needles, fairly soluble in water.

7–Amido–8–hydroxy–quinoline–5–sulfonic acid (III) was obtained by reducing (II) with $SnCl_2$ and HCl by the Witt's method (B. 21, 3472.). Bright orange needles from hot dil. HCl. It does not melt at 310°. It loses its crystall water (1. Mol.) on drying at 110° and takes a more reddish colour.



7–Acetamido–8–hydroxy quinoline–5–sulfonic acid. F. 277° (decomp.).

7–*p*–Nitro–benzoyl–amido–8–hydroxy–quinoline–5–sulfonic acid. F. 297° (decomp.).

7–8–Di–hydroxy–quinoline–5–sulfonic acid was obtained by the diazotisation of (III) and the following decomposition of the diazo compound in warm conc. sulphuric acid.

Yellow needles. F. 302° (decomp.).

7–Amido–8–hydroxy–quinoline was obtained by heating (III) with dil. HCl for six hours at 170°. Slightly coloured prisms. It begins to decompose at 117° and melts at 124°. Hydrochloride: needles. F. 256°.

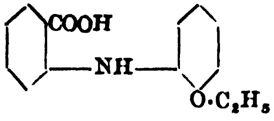
Picrate: reddish brown prisms. F. 205° (decomp.).

7–Acetamido–8–hydroxy–quinoline: colourless needles. F. 177°.

7–Acetamido–8–hydroxy–quinoline–methiodide: yellow needles. F. 195°.

IV. 3–Iodo–4–hydroxy–acridine–1–sulfonic acid.

6′–Ethoxy–diphenylamine–2–carbonic acid (I)



was obtained by condensing *o*-Cl-benzoic acid with *o*-phenetidine. Light yellow needles. F. 160–1.°

4-Ethoxy-(II) and 4-Hydroxy-(III) acridones were obtained by warming the solution of (I) in conc, sulphuric acid for a quarter of an hour on a water bath. The separation of (II) from (III) was effected by the subsequent treatment of the product with dil. NaOH solution.

(II): yellow needles. F. 320° (decomp.). (III): yellow needles. F. 300°.

4-Ethoxy-acridine was obtained by reducing (II) in amyl alcohol with Na. Yellow needles. F. 80°.

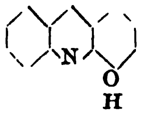
Hydrochloride: yellow needles. F. 220° (decomp.)

Sulphate: yellow needles. F. 250°.

Picrate: yellow needles. F. 255°.

Methyl sulphate: Orange yellow hygroscopic needles. F. 189°.

4-Hydroxy-acridine (IV)

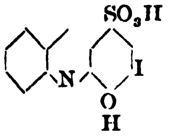


was obtained by reducing (III) in amyl alcohol with Na. Spindle shaped crystalls. F. 117°. Hydrochloride: orange needles. F. 252° (decomp.). Sulphate: orange needles. F. 240°. Picrate: orange needles. F. 215°.

4-Hydroxy-acridine-1-sulfonic acid (V) was obtained by treating (IV) with fuming sulphuric acid (5%) at 8°.

Yellow columns. F. 301° (decomp.).

3-Iodo-4-hydroxy-acridine-1-sulfonic acid



was obtained by iodination of (V) with nascent iodine. Yellow needles. Evolve iodic fumes at 240° and melt at 264° under decomposition.

Besides those mentioned above, several other quinoline derivatives were also synthesised and their physiological effects have been studied by Drs. Hata and Hachiya. The following table denotes their germicidal power for Streptcocci in vitro.

Substance.	Dilution with Serum bouillon.
Pyridine	80
Phenol	100
Quinoline	700
8-Hydroxy-quinoline	500,000
6-Hydroxy-quinoline	1,500
8-Ethoxy-qninoline	600
8-Hydroxy-quinoline-methiodide	5,400
8-Ethoxy-quinoline-methiodide	1,800
8-Hydroxy-quinoline-methyl-sulphate	900
8-Hydroxy-quinoline-5-sulfonic acid	50
7-8-Dihydroxy-quinoline-5-sulfonic acid	200
7-Iodo-8-hydroxy-quinoline-5-sulfonic acid	100

7-Amido-8-hydroxy-quinoline-5-sulfonic acid	1,800
7-Amido-quinoline	<50
7-Acetamido-quinoline	<50
7-Amido-8-hydroxy-quinoline	48,000
5-Amido-8-hydroxy-quinoline	18,000
7-Acetamido-8-hydroxy-quinoline	48,000
5-7-Diamido-8-hydroxy-quinoline	<600
7-Acetamido-8-hydroxy-quinoline-methiodide	13,500
7-Iodo-5-amido-8-hydroxy-quinoline	5,400
Quinoline-cerium chloride	80?
5-Cl-8-hydroxy-quinoline	100,000
5-Iodo-8-hydroxy-quinoline	100,000
5-7-Di-chloro-8-hydroxy-quinoline	100,000
5-7-Di-iodo-8-hydroxy-quinoline	3,000
5-chloro-7-iodo-8-hydroxy-quinoline	1,500
4-Hydroxy-acridine	750?
4-Ethoxy-acridine	750
3-Iodo-4-hydroxy-acridine-1-sulfonic acid	500

EINE MIKROQUANTITATIVE STICKSTOFFANALYSE IN BODEN.

Von Muneari TANAKA.

(*Aus d. Landwirt. Chem. Institut d. Universität Kyoto.*)

(Eingegangen am 10, Okt. 1926.)

Bei der Bestimmung der hauptsachlichen Pflanzennährstoffe im Boden muss man erwägen, dass die hier ausschlaggebenden Mengen meist ausserordentlich gering sind, und dass somit meist die höchsten Aufordungen an die Genauigkeit der chemischen Analyse gestellt werden müssen. Wenn man so z. B. eine Düngung von 7.5 kg. Stickstoff in 75000 kg. Boden geben würde, macht das 1 mg. in 10 gr. Boden. Will man die Analyse auf 1 % genau ausführen, so dürfte der Analysenfehler danach bei Anwendung von 1 gr. Boden ± 0.000001 N nicht überschreiten.

Mitscherlich(1) hat mit seinen Mitarbeitern eine quantitative Stickstoffanalyse nach geringen Mengen ausgearbeitet. Seine Methode besteht in einer Modifizierung des Kjeldahls. Nach den Angaben von Mitscherlich lässt sich der Stickstoff bei geringen Mengen noch auf ± 0.000012 gr. genau bestimmen, jedoch ist seine Methode wegen der vielen Reagenzien die man herstellen muss sehr kompliziert. Ich habe Dubsky's(2) Methode angewandet und konnte dadurch ± 0,00001gr. Gesamtstickstoff von Boden bestimen, und zwar ist es ganz gleich ob dieser Stickstoff in Form von Ammoniak, Nitrat, Nitrit, oder in Form von organischem Stickstoff vorliegt. Die Methode ist folgende :

Zur Kontrolle habe ich zuerst einige bekannte organische Substanzen mit geglühtem Boden oder Kupferoxyd gemischt und verbrannt. Ich habe feststellen können, dass durch dieser Methode der technische Fehler niemals 1% vom enthaltenen Stickstoff überschreitet. z. B.

9.250mg. Brucin ergaben 0.611ccm. N (30° 756mm.) Gefunden 7.14%. Berechnet 7.12%. Demnach beträgt der Fehler nur ±0.02%.

Feiner Boden wird bei 110° zum konstanten Gewicht getrocknet. Davon wurden ganz genau 0.1—0.5gr. Boden auf dem Schiffchen von 3cm. Länge abgewogen und vorsichtig in das Verbrennungsrohr eingeführt. (In diesem Falle kann man Pregl's(3) Methode nicht benützen, da sich zu viele Fehler in den Resultaten vorfinden.) Die Ausführung der Verbrennung ist etwas anders als bei der gewöhnlichen organischen Substauz. Die Stickstoffe in Boden sind meistens ziemlich schwer zu verbrennen. Nachdem die Luft voll kommen verdrägt ist, wird die reduzierte Spirale, Kupferoxydschicht und das Schiffchen weitere 10 Minuten, so dass innerhalb 20 Minuten der Stickstoff vollkommen ausgetrieben ist. Aus vielen Proben lasse ich hier nur einige Analysen, die nach dieser Methode ausgeführt worden sind folgen :

A) 428.40mg. Boden ergaben 0.344ccm. N (30° 755mm.) 0.0844% N Gefunden 441.40mg. desselben Bodens ergaben 0.348ccm. (30° 755m.) 0.0853 % N Gefunden. Fehler ±0.0009%.

B) 217.90mg. Boden ergaben 0.258ccm. N (30° 755mm.) 0.1279% N Gefunden 228.70mg. desselben Bodens ergaben 0.267ccm. N (30° 756mm.) 0.1265% N Gefunden. Fehler ±0.0014%.

LITERATUR :

1) Alf. Mitscherlich und Paul Herz : Eine quantitative Stickstoffanalyse fur sehr geringe Mengen. Landwirt. Jahrb. 1809 u. 1910.

2) J. V. Dubsky : Vereinfachte quantitative Mikroelementaranalyse organischer Substanzen. 1917.

3) Fritz Pregl : Die quantitative organische Mikroanalyse. 1923.

INVESTIGATION ON THE BIILMANN'S QUINHYDRONE ELECTRODE. III.

By Arao ITANO, Satiyo ARAKAWA and K. HOSODA.

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(Received Oct. 27th., 1726.)

The results of the following investigations are reported :

1. Efficiency of the quinhydrone in determination of P_H and titration of various solutions which are commonly used in the soil microbiological investigations.
2. Use of an agar bridge and its study under different working conditions and treatment.

The following table gives the results obtained on the determination of P_H in the solutions, with different chain compared with the H_2–electrode :

TABLE I. Determination of P_H.

	P_H Determination by		
Solutions.	H_2–electrode	Q–C	Q–Qs
A.	3.96	3.61	3.65
B.	6.71	7.01	7.04
C.	6.29	6.45	6.43
D.	7.55	7.22	7.19
E.	6.96	6.70	6.71
F.	7.85	7.64	7.70

Notes : A. Clark's buffer solution ; B. Soil solution ; C. Soil suspension ; D. Nutrient broth ; E. Medium for the Cellulose fermenter ; F. Ashby's solution (mannite).

The table indicates that the quinhydrone electrode gives slightly lower P_H except in the solution B. and in the suspension C. So far as our investigation is concerned, the quinhydrone electrode may be used satisfactorily in all these solutions.

Besides the determination of P_H, the titration was carried out on each solution by using NaOH and HCl. The results obtained are reported in detail accompanied with the graphs in the original paper.

The use of an agar bridge in forming the chain is questioned by some investigators, especially as to the repeated use of the same bridge. The results obtained by the authors show that the agar bridge can be used satisfactorily with very little difference. An old bridge can be used repeatedly with due precautions such as through washing with the distilled water and kept in saturated KCl solution after each experiment.

STUDIES ON THE CARBON-NITROGEN RATIO AND MICROBIOLOGICAL INVESTIGATION OF THE SOIL IN RICE-FIELD.

By Arao ITANO.*

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(Received Oct. 30th., 1926.)

This paper deals with the carbon-nitrogen ratio and microbiological analyses on the soil in rice-field. The experimental plots are located in a portion of the field which has been under cultivation for a few hundred years and the soil is the sandy loam, alluvial type(1). Eleven small plots were used, and the the soil in six representative plots were examined for their carbon(2) nitrogen(3) ratio and for the hydrogen ion concentration(4), previous to the investigation, and the results are shown in the following table:

TABLE I.

Carbon-Nitrogen Ratio and Hyhrogen Ion Concentration.

Plats.	C.	N.	C : N	P_H
1.	1.671%	0·127%	13.2	6.71
3.	1.553	0.130	11.9	6.56
5.	1.556	0.125	12.4	6.69
7.	1.558	0.123	12.7	7.19
9.	1.805	0.117	15.4	7.00
11.	1.304	0.112	11.6	6.88
Mean.	1.575	0.122	12.9	6.84

Each plot was treated differently with or without the addition of various combination of fertilizers which are commonly used on the farm, in this section of Japan. The carbon-nitrogen ratio of each component of the fertilizers is given in Table II.

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* The author was assisted by Satiyo Arakawa and Katsumi Hosoda.

1, 2, 3, & 4. See the original paper.

Table II.

The Carbon-Nitrogen Ratio of Humus Forming Materials used.

Names.	C.	N.	C : N
Compost.	22.41%	1.77%	12.7
Soybean cake.	50.03	9.25	5.4
Cotton seed oil cake.	48.16	7.35	6.6
Genge.*	46.37	2.68	17.3
Straw (barley).	46.49	0.53	87.7
Red clover.	45.30	3.99	11.4

* *Astragalus sinicus.*

The total carbon and nitrogen, added to each plot, were calculated, as shown in Table III.

Table III.

The Carbon-Nitrogen Ratio Provided to each Plot.

	Per cent supplied.		
Plots.	C.	N.	C : N
1.	—	—	13.2
2.	0.14	0.022	6.4
3.	2.13	0.024	88.8
4.	2.58	0.029	89.0
5.	3.61	0.041	88.0
6.	2.20	0.026	84.6
7.	2.65	0.036	73.6
8.	0.04	0.003	13.3
9.	0.11	0.009	12.2
10.	0.14	0.012	11.7
11.	—	—	11.6

The rice was planted on July 3, 1925 and harvested on November 2, 1925. The crop report for each plot was made according to the usual method. The results for the first year are given in the following table :

Table IV.

Amount of Crop Harvested in 1925.

Plots.	Rice in Hask. * (Kilogram per Tan.)	Straw. (Kilogram per Tan.)
1.	Not planted.	
2.	569.9	1143.9
3.	397.6	733.2
4.	386.1	714.0
5.	321.0	604.5
6.	439.3	832.1
7.	360.9	704.7
8.	477.5	834.9
9.	548.6	999.1

10.	523.5	1060.4
11.	455.2	853.7

* Tan = 1/4 of an acre.

After the harvest, the second analyses of the soil in each plot were made for the total carbon and nitrogen, and the hydrogen ion concentration, and the results are given in Table V.

TABLE V.

Carbon-Nitrogen Ratio and Hydrogen Ion Concentration.

Plots.	C.	N.	C : N	P_H.
1.	1.759%	0.240%	7.3	6.94
2.	1.780	0.266	6.7	6.82
3.	1.720	0.273	6.3	7.17
4.	1.678	0.273	6.1	7.11
5.	1.700	0.258	6.6	6.83
6.	1.614	0.238	6.8	6.81
7.	1.660	0.199	8.3	7.06
8.	1.669	0.190	8.8	7.04
9.	1.796	0.181	9.9	6.84
10.	1.559	0.154	10.8	7.08
11.	1.522	0.159	9.6	6.85

On examining the results given here in conjunction with those obtained in Table III, it is interesting to note that the C : N ratio became somewhat constant and lie between 6.1 and 10.8 although each plot was provided with a different ratio at the beginning. In all the plots except Plot 2 of which ratio increased slightly at the end, the ratio became much narrower.

Summary.

From the results obtained thus far, it is premature to draw any conclusion since the investigation began only last year. However several noteworthy facts may be reported at this time :

1. In those plots which received the barley straw alone in various quantity, the amount of crop was inverse ratio to the amount of straw used.

2. The application of the compost and red clover, gave comparatively good crop.

3. It is difficult to find any definite correlation between the crop productivity and the C : N ratio at this time. But on the average, wider the ratio gave the better crop with exception of Plot 2.

4. The C : N ratio in all the plots became somewhat the same while at the beginning, the different ratio was provided.

The microbiological analyses on each plot are in progress in view to find some correlation with the chemical analyses and crop productivity.

STUDIES ON THE BODY-FLUID OF THE SILK-WORM.

By S. Bito.

(Received Nov. 11th., 1926.)

The blood of the insect may be looked upon as the nutrient medium for the growth of the tissues of the body during larval life. It is a physiologically important thing to investigate the nature of the blood of the silk worm on sericulture.

The author has investigated the blood day by day at different stages of its development by chemical analysis customary for routine examination and others.

The summary of the experimental results may be given as follows.

1) The constituents of the blood of silk worms are changed according to its metamorphosis. It is probably owing to moulting, development of silk-gland and development of sexual organs.

2) There is an evident difference between the constituents of the blood of male and female. The female includes more organics than the male, and on the othe.ı hand the male has more inorganics than the fomale.

3) The blood of the silk worm produces the following colour reactions of protein : Biuret reaction, Millon's reaction, Adamkiewicz' reaction, Xanthoproteic reaction, and Ninhydrin reaction. Filtrate of alcohol-ether extract produces Salkowki's reaction. Filtrate free from protein does not reduce Fehling's reagent, but picric test is positive.

4) Two kinds of lipoid, cholesterin and lecithin are contained in the blood of silk worms.

5) When the larva abstains from food, the blood concentrates in the first stage in hunger, and then the blood dilutes, and also when the larva is kept in a cold place the blood concentrates more than in the upper experiment.

6) Of the inorganics, phosphorus and magnesium are very high and kalium, sodium, calcium, silica and sulphuric acid are low. Traces of ferrous and manganese are found and manganese is higher in silk worms than in mammals. The larger parts of phosphorus are combined with organic compounds as lecithin or protein.

7) When the loss of blood is about 15 percent of the body weight, it has bad influence on the health and production of silk matter, which differs in different stages of development.

EINE MIKROQUANTITATIVE KOHLENSTOFF (ODER HUMUS) ANALYSE IM BODEN.

Von Munenari TANAKA.

(*Aus d. Landwirt. Chem. Institut d. Universität Kyoto.*)

(Ausgegangen am 17, Dez., 1926.)

Eine alte, aber sehr genaue Bestimmung des Kohlenstoffgehaltes erfolgte nach G. Logés(1) durch die Verbrennung des Bodens mit Kupferoxyd nach der Makroelementaranalyse. Neulich hat Imai mit seinen Mitarbeitern eine quantitative Kohlenstoffanalyse nach "Verbesserte Chromosäure Methode"(2) ausgeführt. Nach den Angaben von Imai lässt sich der Kohlenstoff in 1$\frac{1}{2}$ Stunden bestimmen, doch da er nur 97% des Kohlenstoffgehaltes angibt, ist die Bestimmung nach seiner Methode keine genaue. Deshalb habe ich Dubsky und Pregl's Methode(3) modifiziert. Durch diese Modifikation gelang es mir, den Gesamtkohlenstoff des Bodens ebenso genau wie nach Loges' Methode zu bestimmen, und zwar noch schneller als nach der Imai's.

Zur Kontrolle der folgenden Methode habe ich zuerst einige reine bekannte organische Substanzen verbrannt. Nach meinen Erfahrungen überschreitet der technische Fehler niemals 0.4 % des enthaltenen Kohlenstoffs: z. B.

7.70 mg. Alizarin ergaben 19.75 mg. CO_2 Ber. 70.1% Gef. 70.0%

Die Ausführung der Analyse ist etwas verschieden, als die bei organischen Substanzen, und es sind besonders die vier folgenden Punkte hervorzuheben:

Erstens. Bei 110°C. getrockneter Boden hat noch sehr viel Wassergehalt, weshalb das gewöhnlich angewandte Mikrocalciumchloridrohr viel zu klein ist. Demnach habe ich ein U Rohr gebraucht, welches dreimal so viel Calciumchlorid enthält als das vorige.

Zweitens. Da der Humus im Boden manchmal nicht einheitlich und gleichmässig vermischt ist, wurde feiner Boden, nachdem er bei 110°C. getrocknet war, im Mörser zerkleinert und innig vermischt. Davon wurden ganz genau 0.02–0.07g. Boden auf dem Schiffchen abgewogen.

Drittens. Der Sauerstoff muss sehr langsam eingeleitet werden, da andernfalls das Wasser ins Natronkalkrohr übergeht. Die Verbrennung ist innerhalb 30 Minuten vollendet.

Viertens. Bei dieser Methode erhält man durch das Wiegen des Bodens nach Verbrennung gleichzeitig den Wert für den Glührückstand. Von vielen

analysen lasse ich hier nur einige, die nach dieser Methode ausgeführt worden sind folgen:

(A) Humus Boden aus Kagoshima

39.45 mg.	Boden	ergaben	12.80 mg.	CO_2	Gefunden	8.83%
33.10 〃	〃	〃	10.60 〃	〃	〃	8.73%
42.30 〃	〃	〃	13.62 〃	〃	〃	8.79%

(B) Humus Boden aus Tottori

37.40 mg.	Boden	ergaben	12.75 mg.	CO_2	Gefunden	8.86%
37.35 〃	〃	〃	12.20 〃	〃	〃	8.90%

LITERATUR

1) G. Loges: Landw, Vers.—Stat, 28, 229 (1883)
2) Industrial & Engineering Chemistry, Vol. 17, No. 1, Jan, 1925.
3) J. V. Dubsky: Vereinfachte quantitative Mikroelementaranalyse organischer Substanzen 1917.
Fritz Pregl: Die quantitative organische Mikroanalyse 1923.

STUDIES ON YEAST-GUM.

By Yoshitaka HASHITANI.

(From the Laboratory of the Dai-Nippon Brewery Company, Tokyo.)

(Received Jan. 9th., 1927.)

Since 1871 when Béschamp(1) gave his report on the studies of yeast-gum many other investigators as Schützenberger(2), Nägeli and Loew(3), Wegner(4), Hessenland(5) and Lindets(6) have published the result of their researches on the subject. There are, however, more or less evidences that they conducted their experiments on impure samples. It was Salkowski(7) who first isolated the substance in pure state as copper salt and gave it the name by which it is now known. K. Oshima(8), who carried out his experiments on the method of Salkowski, stated that the yeast-gum would produce by hydrolysis, plenty of mannose, small quantity of glucose and a trace of methyl-pentose. Euler considers this result is obtained from pure yeast-gum and I am of the same opinion.

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(1) Béschamp: Comptes rend. de l'Ac., 1871, **72**, 337.
(2) Schützenberger: Comptes rend. de l'Ac., 1874, **78**, 493, 698.
(3) Nägeli u. Loew: Journ. f. prakt. Chemie, 1878, **125**, 403; Annalen d. Chemie u. Pharmazie. 1879, **193**, 322.
(4) Wegner: Zeitschr. d. Vereins f. Rübenzuckerind., 1890, **40**, 789.
(5) Hessenland: Zeitshr. f. Rübenzuckerind., 1892, **42**, 671.
(6) Lindets: Comptes rend. de l'Ac., 1891, **112**, 102.
(7) Salkowski: Archiv. f. d. ges. Physiol., 1891, **52**, 554; Chem. Centralbl., 1891, 224.
(8) K. Oshima: Zeitschr. f. Physiol. Chemie, 1902, **36**, 42.

Subsequently this subject has been studied by Meigen & Sreng(9), Euler & Fodor(10), and Harden & Young(11). Then Völts & Bandrexel(12) and Simon(13) reported on the effect of yeast-gum on the digestive power of animal body.

For the determination of yeast-gum a typical method is that of Schönfeld & Künzer(14) and Mayer(15), and more recently that of Ling, Nanji & Paton(16).

It should be remembered that yeast-gum is a kind of the so-called mannane and is one of the leading carbohydrates that constitute the yeast cell. The presence of the gum differs much in quantity according to the variety and condition of yeast but in general it is contained in fairly large quantities. It may safely be said that nearly all the soluble carbohydrates found in yeast cell consist of two substances, namely, yeast-gum and glycogen. The relative quantity of the two in yeast cell varies according to the constituents, the density, acidity, and temperature during fermentation of the nutrient solutions, to the presence or absence of oxygen and carbondioxide, and to the quantity and other physical factors of the yeast employed for fermentation.

Therefore, this quantitative relation of yeast-gum and glycogen is a valuable indication not only of the nutritious conditions of the yeast, but of the treatment it has received. At the same time, the relation indicates the vital condition of fermentation and multiplication of yeast. While glycogen serves as a reserve substance of yeast cell, yeast-gum does not appear to be of any such service in this respect and seems to differ very much from the relation in which higher plants stand to mannane. In general, yeast-gum seems to be present in comparatively large quantities in the types of yeast whose fermenting strength is weak. Thus, yeast-gum might be regarded as a kind of worn-out substance. However, judging from the fact that there is such a close association between yeast-gum and yeast-enzymes as to make it quite difficult to separate them for individual preparation, and especially considering that yeast-gum possesses a property closely relating to invertase, I am driven to the conclusion that yeast-gum has a certain important rôle to play in the physiology of yeast.

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(9) Meigen u. Spreng: Zeitschr. f. Physiol. Chemie, 1908, **55**, 48. Centralbl. f. Bakt., 1908, (2), **21**, 769.

(10) H. Euler u. A. Fodor: Hoppe-Seyler Physiol. Chemie, 1911, **72**, 339.

(11) Harden u. Young: Journ. Chem. Soc., 1912. **101**, 1928; Chemie Centralb., 1913. **1**, 237.

(12) W. Völtz u. A. Bandrexel: Pflügers Achiv., 1905, **107**, 388; Biochem. Zeitschr., 1911, **30**, 457; Ibid, 1911, **31**, 355.

(13) F. Simon: Zeitschr. f. Physiol. Chemie, 1912, **77**, 243.

(14) F. Schönferd & E. Künzer: Wochenschr. f. Br., 1914, **2**, 9.

(15) P. Mayer: Biochem. Zeitschr.. 1923, **136**, 487: through Journ. Inst. Br., 1923. **8**. 667.

(16) A. R. Ling, D. R. Nanji & F. J. Paton: Jour. Inst. Br., 1925. **6**. 316.

Then as the gum has the property to percolate out of yeast cell in consequence of the autolysis of yeast, most likely it will find its way into beer during fermentation and to effect the quality of beer, especially its physical property.

From what has been stated above, it is easy to conclude that the investigation of yeast-gum is of great consequence both from scientific and practical standpoint. For years past I have conducted researches on the "Physiology of Yeast", and will proceed to report here on that part of my studies relating to yeast-gum.

I. METHODS FOR THE DETERMINATION OF YEAST-GUM, AND GLYCOGEN.

In determining glycogen in the yeast, the method hitherto used was a modification of E. Pflüger's process(17) for determining animal constitution, so that the yeast-gum was wholly included in the determination of glycogen. The figures given in former days, therefore, under the name of "Yeast-glycogen", included yeast-gum. It must be noted that under certain conditions, yeast contains greater quantities of gum than glycogen, while our researches have shown that there exists most probably some difference between glycogen and yeast-gum in their physiologycal relation to yeast. From this, it follows that it is necessary to separate these two substances and to effect their distinct determination.

Impressed by that idea I started my investigations years ago and at last devised a process judged suitable for the purpose. It is a happy coincidence interesting to me that the papers by R. Ling, R. Nanji & F. Paton appeared in June, 1925, which came to my hand in September of the year, informed me that they had set about their studies along a similar line and that in many points their investigations agree with mine.

Below will be described the process I have adopted:

About 50 grams of yeast is put in an Erlenmeyer flask to which is added 150c.c. of 66% KOH solution. The mixture is heated for two hours in a hot bath provided with a reflux condenser. After cooling, the mixture is made up to 500c.c. with water and the liquid is cleared by filtration or with a centrifugal separator. Two 100c.c. portions of the clear filtrate are each transferred to a beaker and then alcohol is added. When the alcohol has been concentrated to 60 %, a few drops of saturated NaCl solution is added to precipitate glycogen and yeast-gum. In twelve hours or so, the deposit is filtered off with suction. The resulting precipitate is washed with 60% alcohol, until the filtrate becomes colourless. Washed again two or three times with 95% alcohol, it is dissolved in warm water.

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(17) E. Pflüger: Pflügers Archiv. f. ges. Physiologie, 1909, **129**. 362.

To the one portion of the solution is added HCl to the density of 4% and the mixture is inverted in a hot bath for three hours, and then taken out. After cooling, the solution is neutralized with KOH and made up to 200c.c., of which 25c.c. is used for estimating glucose according to Bertrand's method for determining sugars. The quantity thus obtained was designated as A.

The other portion of the solution is concentrated up to 2% with addition of KOH. By adding further Fehling's solution the copper salt of yeast-gum is precipitated and filtered off about twelve hours later. Well washed with 2% KOH, the precipitate is dissolved in 4% HCl, and washed with HCl of the same strength, till the total volume is made up to about 80c.c. which is inverted in a hot bath for three hours. After cooling, it is neutralized with KOH (which is left off just as the solution begins to get turbid by the precipitation of copper hydroxide) and made up to 100c.c., with water, of which 25c.c. is taken for estimating glucose, according to Bertrand's method. This was designated as B.

The formulae are therefore thus:

$(2A - B) \times 0.9270 =$ Glycogen.
$B \times 0.9472 =$ Yeast-gum.

The coefficient 0.9270 is the figure commonly given to glycogen, while 0.9472 is what we have given to yeast-gum according to my investigations.

II. PREPARATION OF YEAST-GUM.

This was effected mainly on Salkowski's method somewhat modified, details of which are however, omitted here. The yeast-gum thus obtained is a white amorphous and hygroscopic matter which by Fehling's solution forms copper salt but not reduces it. It does not contain nitrogen nor is ash found in determinable quantity. Qualitative reaction, elementary analysis and so forth, prove that the yeast-gum is pure.

III. SPECIFIC ROTATORY POWER OF YEAST-GUM.

Experiments have been made on three different samples of yeast-gum, as to its specific rotatory power, with the Schmidt & Haensch half-shadow polariscope, and the result obtained was as follows:

$[\alpha]_D^{20°} = 90.59°, 90.82°$, and $90.65°$. mean: $90.69°$

IV. FOAMING POWER OF YEAST-GUM.

20c.c. of the aqueous solution of yeast-gum is placed in a bottle for testing hardness of water, and is shaken by hand 100 times. The length of time in which the foam thus produced wholly disappears is compared with that of distilled water similarly treated. It is found that for the 0.05% solution it takes 1–1½ hrs. for the foam to disappear, and 4½–5 hrs. for that of the 0.10% solution, indicating that the time required is 6–8 and 27–30 times as long as that for the distilled water. This shows the strong foaming power of yeast-gum. It may safely be concluded, therefore, that yeast-gum plays an important part in the foaming quality of beer.

V. VISCOSITY OF YEAST-GUM.

The following equation has been obtained from Orth's equation, on the relation between the concentration and viscosity of yeast-gum as experimented by Ostwald's viscosimeter.

$$\log \log \eta = 0.22728 + 0.01380\, x$$

where η is the viscosity of the solution and x is the concentration of yeast-gum.

VI. ELEMENTARY ANALYSIS OF YEAST-GUM.

The elemetary analysis has shown as follows :-

Sample I		0.1839 g.	substance gave	0.2970 g.	CO_2	& 0.1056 g.	H_2O
Sample II							
	a.	0.2342 g.	〃 〃	0.3786 g.	〃	& 0.1276 g.	〃
	b.	0.2324 g.	〃 〃	0.3795 g.	〃	& 0.1292 g.	〃
Sample III							
	a.	0.2423 g.	〃 〃	0.3942 g.	〃	& 0.1388 g.	〃
	b.	0.2555 g.	〃 〃	0.4160 g.	〃	& 0.1386 g.	〃
	c.	0.2241 g.	〃 〃	0.3622 g.	〃	& 0.1286 g.	〃

			C %	H %
Found	Sample I		44.05	6.38
	Sample II	a.	44.09	6.05
		b.	44.54	6.18
	Sample III	a.	44.37	6.36
		b.	44.40	6.03
		c.	44.08	6.38
Cal.	$(C_6H_{10}O_5)_n$		44.44	6.17

VII. HYDROLYSIS OF YEAST-GUM.

When hydrolysed with hydrochloric acid yeast-gum produces a large quantity of mannose, a little of glucose and a trace of methyl-pentose, but nothing of fructose, galactose, pentose and the like.

VIII. DERIVATIVES OF YEAST-GUM.

A. Benzoyl derivative. When an alkali solution of yeast-gum is acted upon by benzoyl-chloride drop by drop, benzoyl derivative of the formula $C_6H_8O_5$ $(C_6H_5CO)_2$ is obtained. The substance thus prepared is whitish amorphous and non-hygroscopic, and insoluble in water, ethyl-methyl alcohol, ether, petroleum-ether, and benzene but soluble in pyridine. Its melting point is 223-225°C and decomposes at 240°C.

B. Acetyl derivative. Various experiments were made on this subject and it was ascertained that the largest yield is obtainable when yeast-gum that was treated several times with glacial acetic acid and then with acetic anhydride and anhydrous sodium acetate. The acetyl derivative thus prepared is a white amorphous matter with the formula $C_6H_7O_5(CH_3CO)_3$ and melts at 178-182°C. It is insoluble in methyl-ethyl alchol, ether and benzene, but soluble in chloroform and pyridine.

IX. THE RELATION OF YEAST-GUM TO ENZYMES AND MICRO-ORGANISMS.

Yeast-gum is not in the least affected by the action of such enzymes as Takadiastase, Kashiwagidiastase, digestine, pancreatine and invertase.

Aspergillus, Cladosporium and Penicillium grow on yeast-gum in some measure and it appears to be the case more or less with Mucor and Oidium. But acetic acid bacteria, yeast and the like do not grow at all on yeast-gum.

X. DEPOLYMERIZATION OF YEAST-GUM BY HEATING WITH GLYCERINE.

5 grams of pure yeast-gum mixed with 40 c.c. of anhydrous glycerine is fully stirred up and heated to 210-220°C. for 4-5 hours. The mixture is washed with water into a beaker. After cooling, aceton of about five times as much is added to it, agitated, and left to settle. The aceton layer is then decanted and the sediment is again dissolved in water, and decoloured with animal charcoal. It is precipitated with alcohol. After repeated purification with water and alcohol, the precipitate is finally washed with ether and dried in a dessicator, yielding 2.5 g. of a substance which possesses the following properties. I consider it as a quite new substance which I named "α-yeast-gum".

A. General Properties of α-yeast-gum. α-yeast-gum is a white amorphous and hygroscopic substance. It is readily soluble in water, but not in alcohol, ether, benzene and aceton. Placed in Fehling's solution, α-yeast-gum forms copper salt insoluble in water, and even when heated it never reduces Fehling's solution.

B. Specific Rotatory Power of α–yeast–gum. I have determined the specific rotatory power of α–yeast–gum, with the following result:

$$[\alpha]_D^{20°} = 79.55°, 80.49°, 80.19°, \quad \text{mean}: 80.08°.$$

C. Elementary Analysis of α–yeast–gum.

The analysis was as follows :–

Sample I. 0.2574 g. Substance gave 0.4188 g. CO_2 and 0.1484 g. H_2O

Sample II. 0.2416 g. 〃 〃 0.3952 g. 〃 〃 0.1410 g. 〃

		C %	H %
Found	Sample I	44.37	6.40
	Sample II	44.61	6.49
Cal.	$(C_6H_{10}O_5)_n$	44.44	6.17

D. Molecular Weight of α–yeast–gum. The molecular weight has been determined with Beckmann's apparatus and according to the freezing-point method. The results were as follows:

Sample I. 1.0544 g. of α–yeast–gum were dissolved in 8.1800 g. of distilled water and it was found that the depression of the freezing-point of this solution was 0.300°.

Sample II. 1.1438 g. of α–yeast–gum were dissolved in 8.2268 g. of distilled water and the depression of the freezing-point of this solution was 0.330°.

Found

Sample I $$M = \frac{100 \times 1.0544 \times 19}{8.1800 \times 0.300} = 816$$

Sample II $$M = \frac{100 \times 1.1438 \times 19}{8.2268 \times 0.330} = 800$$

Mean $M = 808$

Cal. $(C_6H_{10}C_5)_5$ $M = 810$

E. Foaming Power of α–yeast–gum. Experimented as in the case of yeast–gum, it is found that the foaming power of α–yeast–gum is very weak and its 2–3% solution does not differ much from water in this respect.

F. Viscosity of α–yeast–gum. Experiments have been conducted on viscosity of α–yeast–gum as for yeast–gum to ascertain experimental equation, and following equation was obtained.

$$\log \log \eta = 0.22650 + 0.00921\, x$$

In order to compare the viscosity with that for yeast–gum differential coefficient from both these experimental equations works out thus:

$$\frac{d \log \log \eta}{dx} = 0.00921 \cdots\cdots\cdots\cdots \text{(α–yeast–gum)}$$

$$\frac{d \log \log \eta}{dx} = 0.01380 \cdots\cdots\cdots\cdots \text{(yeast–gum)}$$

G. Hydrolysis of α–yeast–gum. Subjected to hydrolysis by hydrochloric acid, α yeast-gum gives just as yeast-gum a large quantity of mannose, a small quantity of glucose and a trace of methyl-pentose.

H. Derivatives of α-yeast-gum. Treated in much the same manner as yeast-gum, α-yeast-gum gives benzoyl and acetyl derivatives, but no methyl derivative has been obtained. This benzoyl derivative $C_6H_8O_5(C_6H_5CO)_2$ is a white amorphous and non-hygroscopic matter which is insoluble in water, ethyl-methyl alcohol, ether, petroleum-ether, and benzene, but soluble in pyridine, chloroform and aceton. Placed in contact with hot alcohol, it is turned into a substance of semi-liquidity. Its melting point is 198–199°C. The acetyl derivatve $C_6H_7O_5(CH_3CO)_3$ is a light amorphous and non-hygroscopic matter of white or light brown colour. It is insoluble in water, ethyl-methyl alcohol, ether, petroleum-ether and benzene, but soluble in pyridine, chloroform and aceton. Its melting point is 158–159°C.

I. Relation of α-yeast-gum to Enzymes and Micro-organisms.

α-yeast-gum is not affected by such enzymes as Takadiastase, Kashiwagidiastase, digestine, pepsine and pancreatine, but is readily affected by different kinds of fungi, yeast, and bacteria.

From what stated above, it will be seen that α-yeast-gum is a matter wholly different from yeast-gum, in regard to its specific rotatory power, foaming power, viscosity, derivatives and relation to micro-organisms.

XI. Yeast–gum as one of the Normal Components of Beer.

Yeast-gum, as stated above, is not affected by the action of various kinds of enzyme, yeast, bacteria, etc., so that the yeast-gum that goes by autolysis of yeast into beer in course of fermentation must be present as a component of beer. Working on that assumption I carried on my researches and succeeded in isolating yeast-gum as copper salt from beer, though the quantity obtained was small. The result was as follows:

		Degree of Balling.	Dextrine in 1000 c.c. beer. (g.)	Yeast-gum in 1000 c c. beer. (g.)	% of yeast-gum for dextrine contents.
Beer soon after Main-fermentation.	A	3.48	17.62	0.02	0.11
	B	3.45	19.04	0.02	0.11
	C	3.45	17.79	0.02	0.11
Beer soon after After-fermentation.	A	2.48	17.02	0.04	0.24
	B	2.90	16.80	0.09	0.54
	C	2.30	17.47	0.09	0.51
	D	2.60	15.91	0.05	0.31

From the above table it will be seen that though of very little content, yeast-gum is always found in beer as its component. Hitherto in the analysis of beer yeast-gum has usually been regarded as dextrine and determined as such. However yeast-gum though present in a very limited quantity, affects the qualtiy, especially physical property of beer, so that its presence in beer, however small in quantity, can not be ignored. Further, as shown in the foregoing table, yeast-gum grows in quantity during after-fermentation up to 2–4 times in the beer that has gone through its main-fermentations. From this fact it may easily be judged that during after-fermentation the yeast by its autolysis exudes various substances in addition to yeast-gum and exerts no small effect on the quality of beer.

XII. Change of Yeast-gum in the Yeast Cell placed in the State of Starvation.

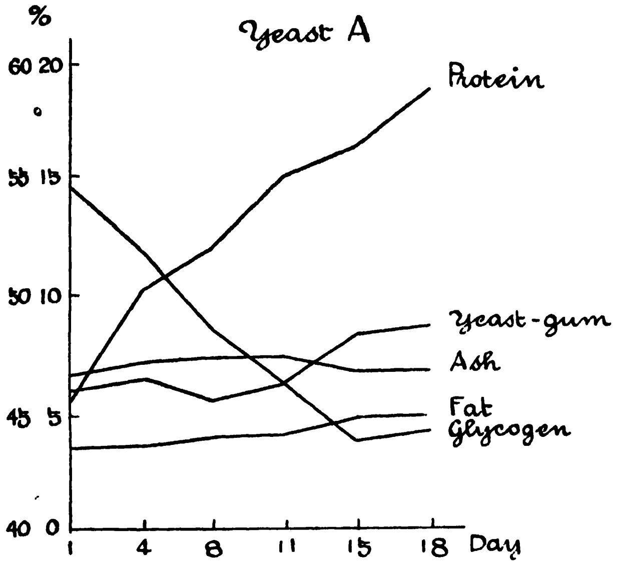
No definite solution has yet been given as to what physiological rôle yeast-gum may play on yeast. In order to attempt this I subjected the yeast long to the state of starvation, depriving it from any nutritive substances, and I then studied how with the change of all other components in the yeast cell during the starvation, the content of yeast-gum goes to vary, and tried also to find indirectly something of the physiological relations in which each component of the yeast cell stands to the yeast.

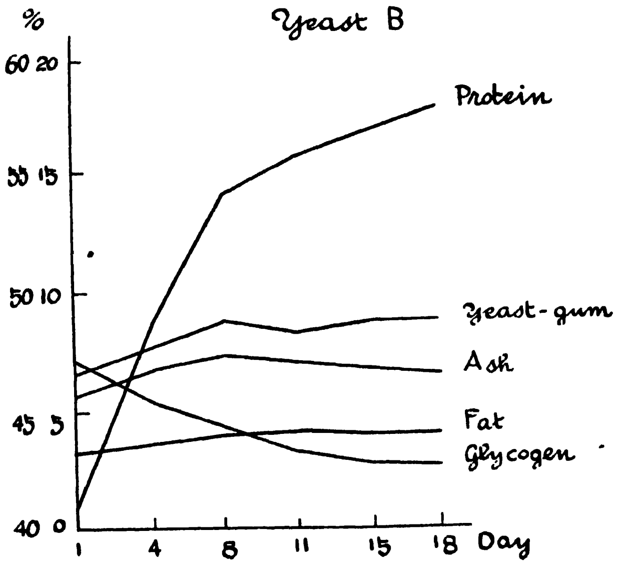
With that object in view, I took a few litres of muddy yeast and washed the sample several times with the clean service-water filtered through a porous pot and let it precipitate the yeast in a large volume of the service-water. It was then stored at a cold place at 2–3°C. Every other day the water was renewed and at a few days' intervals about a litre of the muddy yeast was taken and put through cloth strainer to remove resin. Well washed with water, it was fully filtered with suction to remove the moisture to the atmost, and this sample was used for analysis. The results are tabulated below:

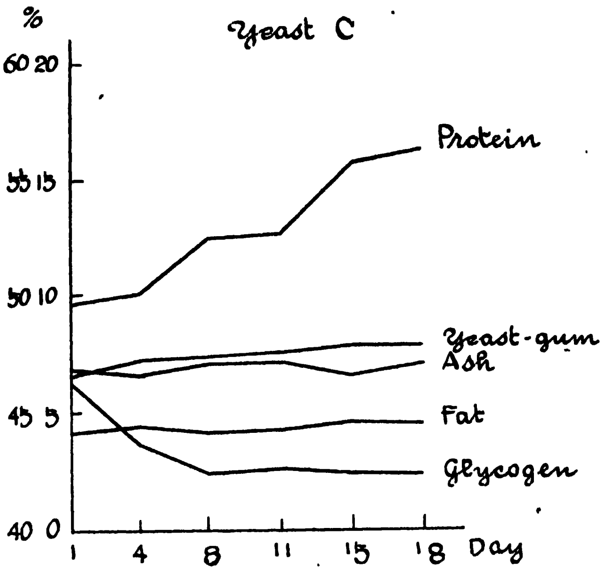
		1st day	4th day	8th day	11th day	15th day	18th day
Yeast A	Sp. gr.	1.5447	1.5133	1.5013	1.4878	1.4827	1.4739
	Protein	45.35	50.14	51.76	54.94	56.34	58.98
	Glycogen	14.48	11.81	8.48	6.31	3.82	4.27
	Yeast-gum	5.81	6.23	5.63	6.03	8.17	8.43
	Fat	3.546	3.772	4.043	4.097	4.901	4.906
	Ash	6.491	6.943	7.125	7.179	6.823	6.836
Yeast B	Sp. gr.	1.4769	1.4852	1.4965	1.4733	1.4712	1.4628
	Protein	40.58	49.39	54.14	55.71	56.93	58.01
	Glycogen	6.96	5.52	4.55	3.39	2.86	2.79
	Yeast-gum	6.66	7.71	8.84	8.19	8.91	8.85
	Fat	3.429	3.676	4.262	4.074	4.042	4.009
	Ash	5.749	6.577	7.143	6.913	6.714	6.507

Yeast C	Sp. gr.	1.5227	1.4832	1.4827	1.4727	1.4745	1.4724
	Protein	49.63	49.94	53.57	53.70	55.75	56.39
	Glycogen	6.18	3.70	2.60	2.70	2.54	2.33
	Yeast-gum	6.66	7.20	7.35	7.55	7.97	7.80
	Fat	4.121	4.454	4.092	4.150	4.660	4.564
	Ash	6.716	6.655	7.030	6.992	6.659	6.949

which is graphically represented in the following:







As shown in the graph, except glycogen all the other components have a tendency to grow in percentage, according to number of days during which the yeast was left under starving conditions. When it is remembered, however, that in the present experiments the yeast was cut off from external nutritive substance, there can be no possibility of all the components gaining in quantity during the period. The proper explanation seems to be that the yeast consumes some of its components through autolysis, so that a component found on analysis to have gained in percentage must have done so at the expense of some other components. A question then naturally arises. Is there no change in absolute quantity? Most probably there is a little decrease. There have recently appeared some reports on the degeneration of yeast, attributing it to the gradual accumulation of protein and fat. This is, however, misleading, for if the components other than those two are left out of consideration, it will merely mean the increase of percentage alone, but not of an absolute quantity.

Now protein is the most important component in the life of yeast cell, and as its content is least subject to change, I have taken it as standard of comparison with the index number of 100. To this I have proportioned the other components and worked out what may be regarded as a kind of absolute quantities.

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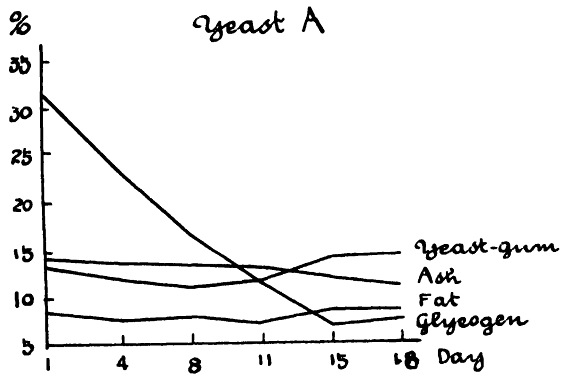
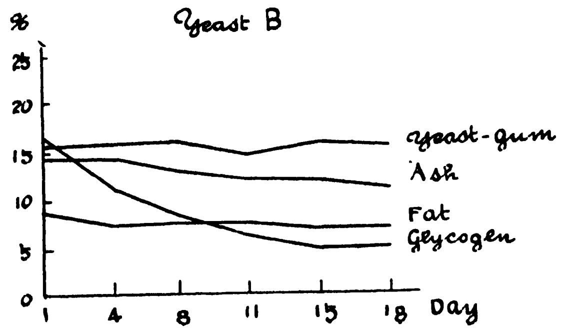
Y. HASHITANI:- Studies on Yeart-gum(Vo 3. No.1)

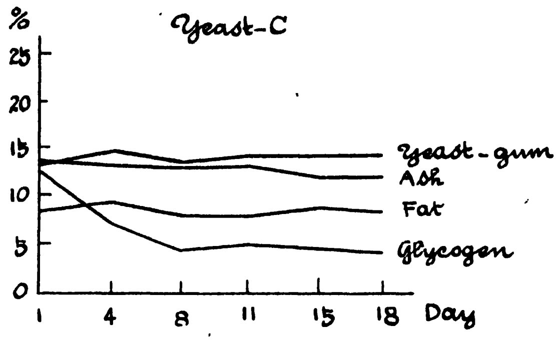
Page	Line	Error	Correct
3	1	small	a small
4	21	physiologycal	physiological
"	23	to effect	effect
"	"	determination	determinations
"	29	agree	agreed
5	22	(2A – B)	(A – B)
7	9	and then	was then treated
10	5	main-fermentations	main-fermentation
"	20	precipitate the yeast in	precipitate in
"	25	atmost	utmost
"	28	Sp gr 1.5447	Sp.gr. 1 5147

		1st day	4th day	8th day	11th day	15th day	18th day
Yeast A	Protein	100.00	100.00	100.00	100.00	100.00	100.00
	Glycogen	31.93	23.55	16.38	11.49	6.78	7.24
	Yeast-gum	12.81	12.43	10.88	10.98	14.50	11.29
	Fat	7.819	7.523	7.811	7.457	8.699	8.318
	Ash	14.379	13.847	13.765	13.030	12.110	11.590
Yeast B	Protein	100.00	100.00	100.00	100.00	100.00	100.00
	Glycogen	17.15	11.41	8.40	6.09	5.02	4.81
	Yeast-gum	16.41	15.93	16.33	14.70	15.65	15.25
	Fat	8.449	7.596	7.872	7.312	7.099	6.907
	Ash	14.167	14.592	13.194	12.409	11.793	11.211
Yeast C	Protein	100.00	100.00	100.00	100.00	100.00	100.00
	Glycogen	12.45	7.41	4.85	5.03	4.56	4.13
	Yeast-gum	13.42	14.42	13.72	14.06	14.30	13.83
	Fat	8.303	8.918	7.638	7.728	8.358	8.093
	Ash	13.533	13.326	13.123	13.020	11.941	12.323

which is shown graphically in the following:







From the graph we learn that although, according to the nutritious conditions of the yeast, the quantity of each component somewhat changes, yet glycogen alone markedly decreases in any kind of yeast. Thus, it is evident that for yeast during its starvation glycogen is the only carbon source and that it derives its energy from the decomposition of glycogen.

The presence of yeast-gum in yeast is generally in inverse proportion to that of glycogen, being greater when the latter is smaller, but this disparity is very little. It may, therefore, be concluded that yeast-gum lacks its property as a reserve substance, for if otherwise, the yeast containing only a little of glycogen would have to consume its own yeast-gum instead of glycogen, but this goes counter to the fact. Nor does fat present any material variation, though it is subject to slight decrease in yeast with little glycogen. The content of ash in yeast cell somewhat decreases during starvation.

I wish to express my thanks to Dr. K. Makoshi for his valuable advice and kindness given during the present study.

THE INFLUENCE OF ULTRA-VIOLET LIGHT UPON THE GROWTH OF ANIMALS.

By Kozo SUZUKI and Tadashi HATANO.

(Imperial Zootechnical Experiment Station, Chiba, Japan.)

(Received Jan. 10th., 1927.)

The authors conducted a series of experiments to see the influence of ultra-violet light upon the growth of animals.

1. EXPERIMENTS WITH WHITE RATS.

6 young albino rats, (3♂ and 3♀) almost of the same and approximately of the same weight were divided into 3 pairs, and were kept in wire cages, placed about one metre apart each other in a photographic room where no direct light was admitted, except the rays coming through the small red-glass window of the room.

Their ration was composed as follows: powdered white of egg 16%, starch 67 %, butter fat 12 %, and McCollum's salt (No. 185) 5 %, to which ration was added "oryzanin" in the proportion of 0.5c.c. per rat each day.

The ultra-violet light was radiated by a "Silectra-Standard" quartz mercury-vapor lamp provided with a filter.

The first pair was taken out of the room and irradiated for 15 minutes daily at a distance of 40cm. from the mercury lamp; the second pair was always kept in the dark, but their diet was irradiated for 15 minutes daily at a distance of 10cm. from the lamp; while the third pair was kept in darkness and received non-irradiated diet as a control.

After 56 days, the following results were obtained :-

	Days		0	7	14	21	28	35	42	49	59
1st Pair	Body-weight in grms.	male	28	45	72	95	117	133	150	163	179
(Irradiated Body……)		female	31	48	74	93	107	118	130	140	150
		average									164.5
2nd Pair			32	52	68	91	100	110	125	135	153
(Irradiated Diet ……)	"	"	25	30	40	60	73	78	86	97	105
											129.5
3rd Pair			28	33	42	60	64	73	87	97	100
(Control)	"	"	29	33	36	48	49	58	78	87	89
											94.5

We see from the above results that the rats of the first pair which were directly irradiated gained in body weight most rapidly. The second pair which received the irradiated diet showed also much better increases in body weight than the control rats of the third pair which were kept in absolute darkness and received no irradiation. No pathological abnormality could, however, be observed in any of them.

2. EXPERIMENTS WITH CHICKENS, WHITE LEGHORN.

Young chickens were divided into two groups and kept under ordinary diffused lights. The one group consisting of 23 chickens was irradiated for

30 days from the next day of hatch 15 minutes daily, at a distance of 40cm. from the lamp. The ration consisted of: 55 % rice refuse, 7 % wheat, 1 % millet, 25 % fish meal, 3% bone meal, and finely chopped green vegetables.

The control, non-irradiated chickens, did not only grow uniformly, but two of them succombed from malnutrition in 6th and 10th day, respectively; while the irradiated ones grew very well, and none of them died during the experiment. Their average body weights were as follows:-

	0	1	2	3	4	5	weeks
Irradiated Group	38.06	41.93	61.06	94.65	134.76	177.34	grams
Control Group	34.15	37.95	59.16	72.66	103.21	134.71	grams

3. Experiments with Rabbits.

Eight young rabbits were divided into two groups and kept under diffused day light. The ration consisted of: 35% barley, 35% wheat, 30% soy bean cake, besides green vegetable and "tofu" refuse.

The treatment was exactly same with that of the chickens. The irradiation was commenced from 56 days after birth and lasted for 39 days. One of the control rabbit died from malnutrition; while the irradiated animals were all healthy. The average body weights were as follows:-

	0	1	2	3	4	5	weeks
Irradiated Group	215	305	477.5	631.2	762.5	971.3	grams
Control Group	215	282.5	431.0	576.2	680.0	901.0	grams

The above results show that the effect of irradiation was very remarkable, even when the animals were kept under diffused lights. Therefore, the ultra-violet rays present in ordinary day light seem to be far from sufficient for the vigorous development of young chickens. This observation will perhaps throw a certain new light upon the improvement of poultry-raising industry.

I. A. R. I. 75.

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